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Updated Carcinogenicity Assessment for Tetrachloroethylene (Perchloroethylene PERC, PCE): Addendum to the Health Assessment Document for Tetrachloroethylene

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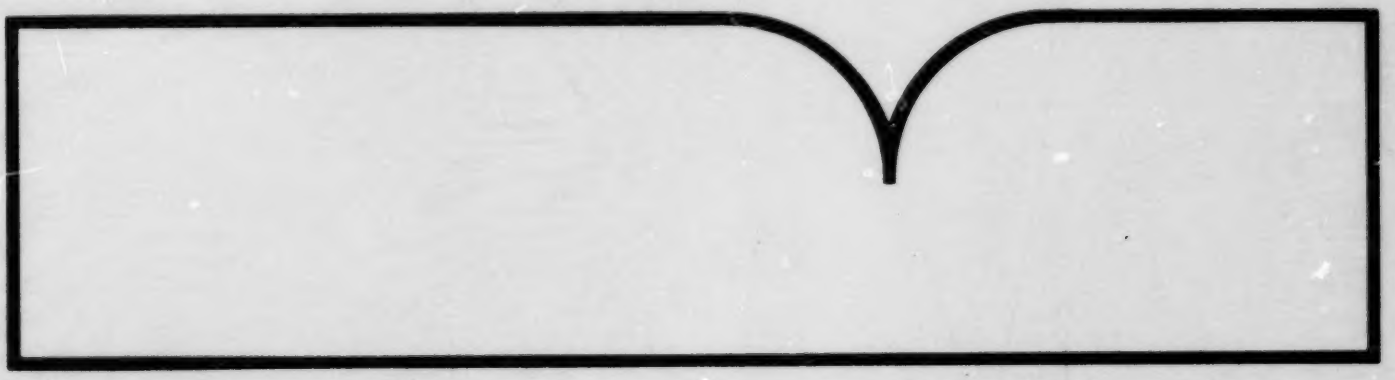

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Addendum to the Health Assessment Document for Tetrachloroethylene (Perchloroethylene)

Review Draft

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Updated Carcinogenicity Assessment for Tetrachloroethylene (Perchloroethylene, PERC, PCE)

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The Office of Health and Environmental Assessment has prepared this addendum to serve as a source document for EPA use. This addendum updates EPA's July 1985 Health Assessment Document for Tetrachloroethylene (Perchloroethylene, PERC, PCE) by providing a review of the findings of the draft National Toxicology Program (NTP) Technical Report on the Toxicology and Carcinogenesis Studies of Tetrachloroethylene in F344/N rats and B6C3F1 mice (inhalation studies). The addendum discusses the ways in which the data impact the assessment of the weight-of-evidence for the carcinogenicity of PCE, and uses relevant data to develop a revised unit risk estimate for inhalation exposure to PCE.

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ADDENDUM TO THE HEALTH ASSESSMENT DOCUMENT

FOR TETRACHLOROETHYLENE (PERCHLOROETHYLENE)

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for Tetrachloroethylene (Perchloroethylene, PERC, PCE)

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Office of Research and Development
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PREFACE

The Office of Health and Environmental Assessment has prepared this addendum to serve as a source document for EPA use. This addendum updates EPA's July 1985 Health Assessment Document for Tetrachloroethylene (perchloroethylene, PERC, PCE) by providing a review of the findings of the draft National Toxicology Program (NTP) Technical Report on the Toxicology and Carcinogenesis Studies of Tetrachloroethylene in F344/N rats and B6C3F1 mice (inhalation studies). The addendum discusses the ways in which the data impact the assessment of the weight-of-evidence for the carcinogenicity of PCE, and uses relevant data to develop a revised unit risk estimate for inhalation exposure to PCE.

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1. SUMMARY AND CONCLUSIONS

In the July 1985 Health Assessment Document for Tetrachloroethylene (perchloroethylene, PERC, PCE), the U.S. Environmental Protection Agency's Office of Health and Environmental Assessment (OHEA) summarized the pertinent evidence for the carcinogenicity of PCE, based primarily on the results of a 2-year bioassay conducted by the National Cancer Institute (NCI, 1977). In that study, PCE induced a statistically significant increase in the incidence of hepatocellular carcinomas in both high- (1072 and 772 mg/kg/day) and low-dose (536 and 386 mg/kg/day) male and female B6C3F1 mice administered PCE by gavage for a period of 78 weeks.

In a 1985 inhalation bioassay conducted by the National Toxicology Program (draft report approved by the Board of Scientific Counselors), groups of 50 male and 50 female F344/N rats and B6C3F1 mice were exposed to PCE (99.9% pure), 6 hours/day, 5 days/week, for 2 years. The exposure concentrations used were 0, 200, and 400 ppm for rats and 0, 100, and 200 ppm for mice. At the 200 and 400 ppm exposure concentrations, PCE induced a marginally statistically significant increase of mononuclear cell leukemia in male rats. PCE also induced an increased incidence of renal tubular cell adenomas and/or carcinomas (combined) in male rats. While not statistically significant, there was a dose-related trend of these rare tumors. In female rats at both the 200 and 400 ppm exposure concentrations, statistically significant increased incidences of mononuclear cell leukemia were observed. In male and female B6C3F1 mice, PCE induced a statistically significant increased incidence of hepatocellular carcinomas at both the 100 and the 200 ppm exposure concentrations. Although mutagenicity studies of PCE have produced inconclusive or negative results, PCE epoxide, a reactive metabolite of PCE, has been found to be mutagenic.

The NTP (1985, draft) inhalation bioassay demonstrated that PCE can induce carcinogenic effects in both rats and mice through inhalation exposure. The earlier NCI (1977) bioassay provided positive evidence of hepatocellular carcinomas in mice administered PCE by gavage. These findings, together with the supporting evidence regarding the PCE epoxide (a presumed reactive metabolite that has been shown to be mutagenic), constitute a "sufficient" level of evidence for the carcinogenicity of PCE in animals, using the EPA's Proposed Guidelines for Carcinogen Risk Assessment. The evidence in rats and mice, together with the inconclusive evidence in humans as reported in the 1985 Health Assessment Document for Tetrachloroethylene, results in the placement of PCE in EPA's weight-of-evidence Group B2, meaning that it should be considered a probable human carcinogen.

OHEA is aware of three issues related to the NTP (1985, draft) inhalation bioassay. These issues are raised in a document presented to EPA by the Halogenated Solvent Industry Alliance (HSIA), and focus on reported carcinogenic responses in male F344 rats, as well as on certain aspects of the conduct of the bioassay. The validity of a reported marginally statistically significant increased incidence of mononuclear cell leukemia in male F344 rats is in question. The finding is dependent on the criteria used for "staging" the leukemia and the approach used in assessing the variable incidence in the untreated rats. The reasonableness of these approaches, as used in data interpretation, is at issue. Also at issue is the significance of a reported finding of a low incidence (not statistically significant) of rare tubular cell adenomas or carcinomas in male F344 rats. Lastly, alleged deficiencies in animal handling and identification raise doubts about the reliability of the reported marginal and low-level incidences of tumors in male rats.

The impact of the issues regarding the findings in male F344 rats relative

to a weight-of-evidence evaluation is to add to the multiplicity of evidence if the findings are considered valid; on the other hand, if the findings are considered uncertain, the remaining evidence in male and female mice and female rats is likely to be judged "sufficient" according to EPA's Proposed Guidelines for Carcinogen Risk Assessment.

In this addendum, an inhalation unit risk estimate for PCE is developed using data from the NTP (1985, draft) inhalation bioassay--the first lifetime inhalation study to show evidence of the carcinogenicity of PCE. Carcinogenic responses were observed in both rats and mice. These data provide a basis for directly estimating the risk of PCE by the inhalation route, and an opportunity to test the reasonableness of the previous inhalation risk estimate, which was developed using gavage data from the NCI bioassay (1977).

It is generally recognized that the carcinogenic potential of PCE resides in its biologically reactive metabolites, such as the short-lived, reactive PCE epoxide intermediate, rather than the parent PCE compound itself. The rate of metabolism of PCE is dose-dependent--meaning that the effective dose of active metabolite is not proportional to the administered dose of PCE--and proceeds according to Michaelis-Menten kinetics. Covalent binding and hepatotoxicity of PCE are directly proportional to metabolized dose. The tumorigenic response is also assumed to be directly related to metabolized dose.

The ingestion and inhalation unit risk estimates reported in the 1985 Health Assessment Document for Tetrachloroethylene were extrapolated from a gavage cancer bioassay study in mice. Metabolism and kinetic information were incorporated into these estimations, expressing dose in terms of the amount metabolized, or a "metabolized dose." Thus, the inhalation unit risk estimate for PCE reported in the 1985 document was based on tumor incidence data from a gavage study and the assimilated dose metabolized, accomplished by incorporating

metabolism and kinetics information for both animals and humans. On this basis, the inhalation unit risk is $4.8 \times 10^{-7}/\mu g/m^3$. The unit risk for ingestion of 1 µg PCE/L in drinking water is 1.5×10^{-6}.

The new unit risk estimate for inhalation also utilizes the metabolized dose-tumor incidence relationships derived from pertinent exposure concentration dose-metabolism relationship data in both animals and humans. The linearized multistage model is used for the low-dose extrapolation. The details of this procedure are presented in the 1985 Health Assessment Document for Tetrachloroethylene. The typical uncertainty associated with extrapolating dose from animals to humans is reduced by the consideration of actual human exposure concentration dose-metabolism relationship data, where urinary trichloroacetic acid is taken as an index of the amount of PCE metabolized, in addition to the use of comparable animal data. The inhalation unit risk estimates calculated on the basis of animal inhalation carcinogenicity studies (rats and mice) are comparable to that previously derived from the gavage carcinogenicity data in mice. The upper-bound estimates of the incremental cancer risk due to lifetime exposure to 1 $\mu g/m^3$ of PCE in air are calculated for six sets of dose-tumor incidence data by three methods. The ranges of the six estimates under each of the three methods are as follows: (a) 2.9×10^{-7} to $9.5 \times 10^{-7}/\mu g/m^3$; (b) 2.9×10^{-6} to $1.1 \times 10^{-5}/\mu g/m^3$; (c) 9.6×10^{-7} to $3.6 \times 10^{-6}/\mu g/m^3$.

The first range of estimates is calculated on the basis of metabolized doses that are derived directly from a single-exposure balance study (assuming that the total amount metabolized over a 72-hour period from a one-time exposure is comparable to the steady-state metabolized dose under the NTP multiple-exposure cancer bioassay study). The second and third range of estimates are calculated on the basis of metabolized doses that are derived from a physiolog-

ically-based pharmacokinetic (PB-PK) model. Such models, which piece together the relevant physiological and biochemical information, can be useful tools for predicting absorption, distribution, biotransformation, and elimination of chemicals across species when sufficient data are available. However, until more data become available, EPA's Carcinogen Assessment Group (CAG) recommends that the first range of estimates be used to represent the inhalation risk for PCE, since there are uncertainties with parameters used in the PB-PK model that were estimated from the same empirical data set used in the direct estimation, and the model cannot be considered to give a better estimate. That is, the revised upper-bound estimate of the incremental cancer risk due to lifetime exposure of 1 $\mu g/m^3$ of PCE in air ranges from 2.9×10^{-7} to 9.5×10^{-7}. This unit risk estimate is applicable only for low-level exposures where the relationship between the ambient air concentration and metabolized dose is linear. The upper-bound nature of these estimates is such that the true risk for humans is not likely to exceed these values and may be lower. Expressed in terms of relative potency, PCE ranks in the lowest quartile among 55 suspect or known carcinogens evaluated by the CAG.

2. INTRODUCTION

The July 1985 Health Assessment Document for Tetrachloroethylene (perchloroethylene, PERC, PCE) (U.S. EPA, 1985) summarized the evidence that PCE administered by gavage induced malignant tumors of the liver in both male and female B6C3F1 mice (NCI, 1977). The Health Assessment Document also cited the results of several epidemiologic studies, which were characterized as providing inconclusive findings. According to the Agency's Proposed Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1984), the carcinogenic evidence available as of July 1985 for PCE in animals was judged to be limited, and the epidemiologic data inconclusive. The overall weight-of-evidence for the carcinogenicity of PCE was classified as belonging in EPA Group C, meaning that PCE should be considered a "possible" human carcinogen. Quantitative human unit risk estimations for ingestion and inhalation were developed using the NCI mouse gavage study, relevant metabolism and kinetics data, and the linearized multistage model for low-dose extrapolation.

Since publication of the July 1985 Health Assessment Document for Tetrachloroethylene, the National Toxicology Program inhalation bioassay draft report (NTP, 1985) on PCE carcinogenicity in rats and mice has become available. The study findings have been audited and validated for technical accuracy, and the report has been peer-reviewed by the NTP Board of Scientific Counselors. Male F344/N rats in the study showed a statistically significant increased incidence of mononuclear cell leukemia and a dose-related trend for rare renal tubular neoplasms. Female F344/N rats showed statistically significant incidences of mononuclear cell leukemia. In addition, PCE induced a statistically significant increased incidence of hepatocellular carcinomas in both male and female B6C3F1 mice.

This addendum reviews the new NTP (1985, draft) inhalation bioassay of PCE in detail, and discusses the ways in which the data impact the assessment of the weight of evidence for the carcinogenicity of PCE. This addendum also includes a section on unit risk estimation which incorporates relevant inhalation metabolism and kinetics data.

3. REVIEW OF THE NATIONAL TOXICOLOGY PROGRAM INHALATION CARCINOGENICITY BIOASSAY (NTP, 1985)

A 2-year inhalation cancer bioassay of PCE in F344/N rats and B6C3F1 mice has been performed by Battelle Pacific Northwest Laboratories, under the sponsorship of the National Toxicology Program (NTP, 1985). The study provides positive evidence for the carcinogenicity of PCE in both of the above species. A description of the various aspects of the study follows.

The study utilized high-purity PCE (99.9%) obtained from the Dow Chemical Company. The purity and identity analyses of the PCE were performed by the Midwest Research Institute. The PCE was stabilized with n-methylmorpholine (53 ppm) to prevent decomposition. The PCE was then vaporized at 100°C to 110°C, diluted with air, and introduced into the inhalation chambers. The concentrations in the chambers were monitored 8 to 12 times per exposure period by means of a Hewlett-Packard 5840A gas chromatograph equipped with a flame ionization detector.

3.1. ANIMALS STUDIED

The 8- to 9-week-old male and female F344/N rats and B6C3F1 mice used in this study were obtained from the Charles River Breeding Laboratories. Groups of 50 rats of each sex were exposed to air containing PCE at concentrations of 0 (chamber control), 200, or 400 ppm, 6 hours/day, 5 days/week for 103 weeks. Groups of 50 mice of each sex were also exposed to PCE at 0, 100, and 200 ppm. These doses were selected based on the results of an earlier 13-week study in which incidence of death at 1,600 ppm and incidence of liver lesions at lower concentrations had been observed for rats. Incidence of death at 1600 ppm, and of hepatic and renal lesions at lower concentrations, were observed for mice. Rats and mice were housed individually. The animals were given a diet (NIH07

Open Formula) and water freely, except during the exposure period, when water was available.

All animals were observed two times per day. Clinical signs were recorded once per month. Body weights were recorded once each week for the first 13 weeks of the study and once per month thereafter. Mean body weights were calculated for each group. Moribund animals, as well as animals that survived to the end of the study, were killed. A necropsy was performed on all animals. Vital organs were examined for any grossly visible lesions. Tissues were preserved in 10% neutral buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin, with other stains being used as needed. The tissue slides were examined microscopically to determine pathology.

3.2. RESULTS OF THE RAT STUDY

Figure 3-1 presents the growth curves for male and female rats exposed to PCE by inhalation for 2 years. The mean body weights of PCE-exposed male and female rats were comparable with those of controls.

Survival data for rats in the 2-year inhalation study are given Table 3-1 and Figure 3-2. The survival of male rats exposed to 400 ppm was significantly reduced (control, 23/50; 200 ppm, 20/50; 400 ppm, 12/50) but not that of females (control, 23/50; 200 ppm, 21/50; 400 ppm, 24/50). Most of the unscheduled deaths (87%) in the 400 ppm dose group occurred late in the study (week 82 or later) and may have been related to the high incidence of mononuclear cell leukemia.

Table 3-2 presents the incidence of mononuclear cell leukemia in male and female rats exposed to 200 and 400 ppm PCE. Incidences of mononuclear cell leukemia in male rats were marginally statistically significant: control, 28/50; 200 ppm, 37/50 ($p = 0.046$); 400 ppm, 37/50 ($p = 0.046$). In female rats, the incidences of mononuclear cell leukemia were also statistically significant: control, 18/50; 200 ppm, 30/50 ($p = 0.014$); 400 ppm, 29/50 ($p = 0.022$).

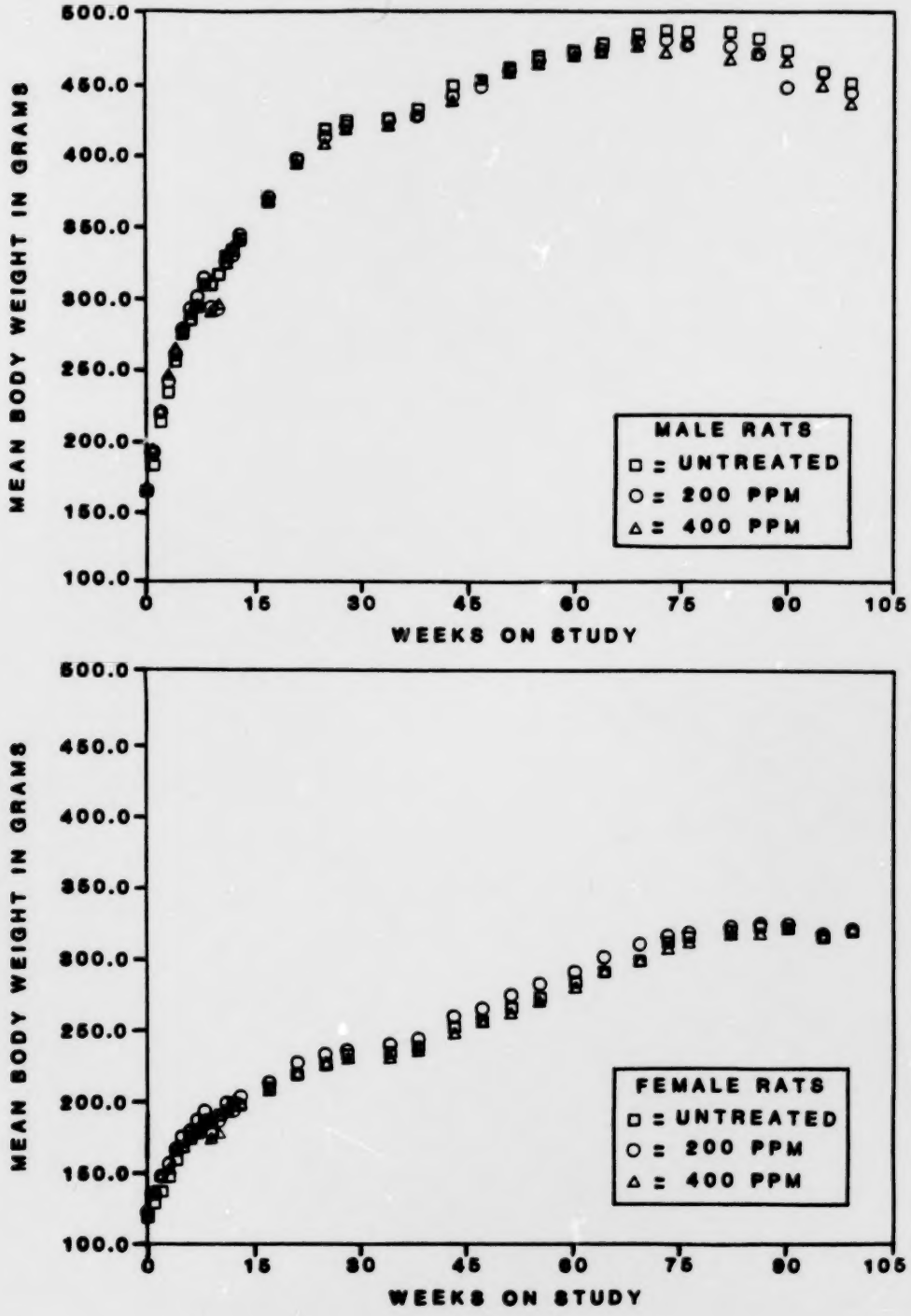

Figure 3-1. Growth curves for rats exposed to PCE by inhalation for 2 years.

SOURCE: NTP, 1985.

TABLE 3-1. SURVIVAL OF RATS
IN THE NTP 2-YEAR INHALATION BIOASSAY OF PCE

Group	Control	200 ppm[a]	400 ppm
Males			
Animals initially in the study	50	50	50
Nonaccidental deaths	27 (54%)	30 (60%)	38 (76%)
Died during termination period	0	1 (2%)	1 (2%)
Killed at termination	23 (46%)	19 (38%)	11 (22%)
Females			
Animals initially in the study	50	50	50
Nonaccidental deaths	27 (54%)	29 (58%)	26 (52%)
Killed at termination[b]	23 (46%)	21 (42%)	24 (48%)

[a]1 ppm is equivalent to 6908 $\mu g/m^3$.
[b]Terminal kill period: week 104.

SOURCE: NTP, 1985.

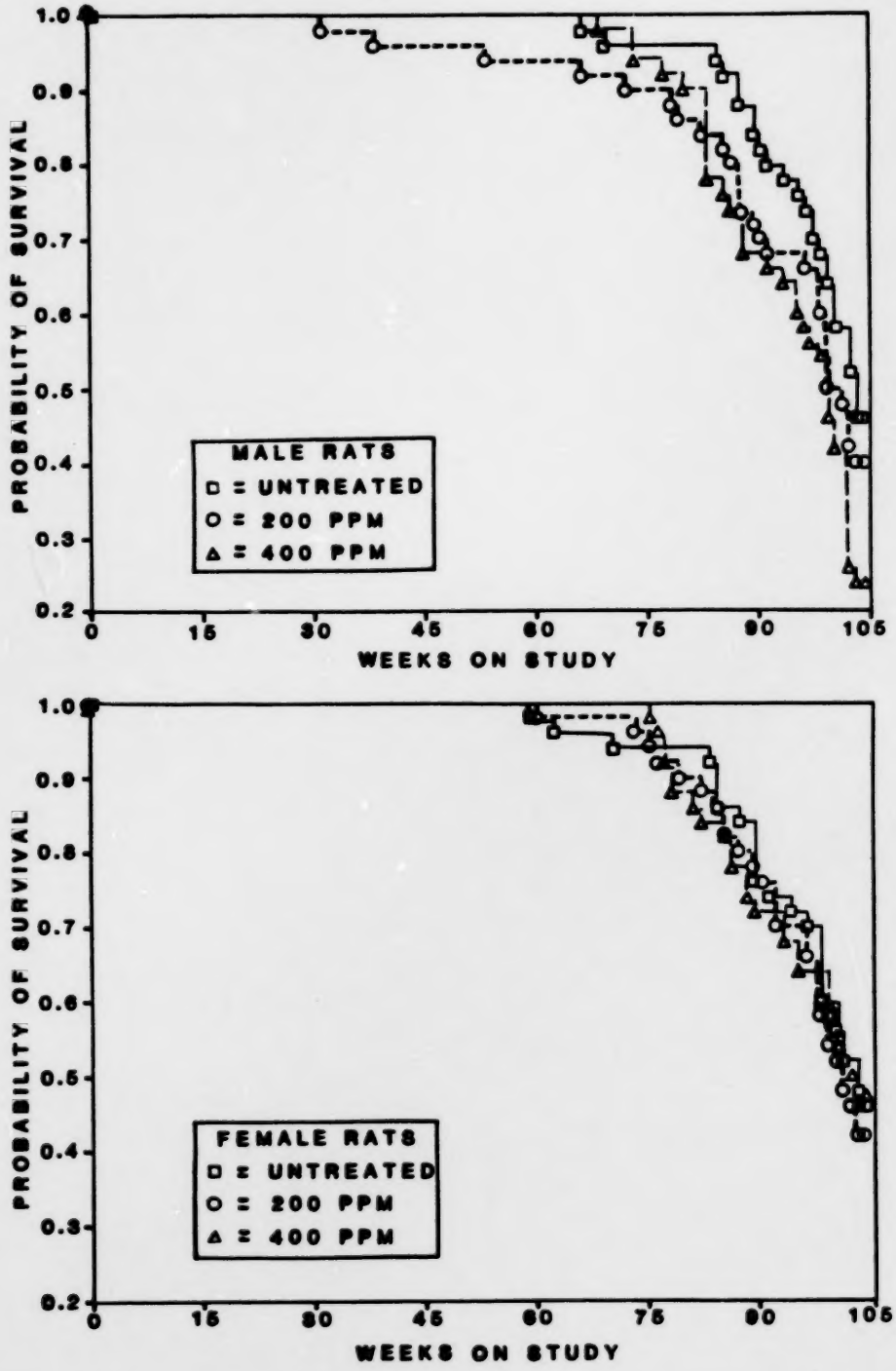

Figure 3-2. Kaplan-Meier survival curves for rats exposed to PCE by inhalation for 2 years.

SOURCE: NTP, 1985.

TABLE 3-2. MONONUCLEAR CELL LEUKEMIA IN RATS
EXPOSED TO PCE BY INHALATION

Group	Control	200 ppm[a]	400 ppm[a]
Males			
Mononuclear cell leukemia	28/50 (56%)	37/50 (74%) p = 0.046	37/50 (74%) p = 0.046
Females			
Mononuclear cell leukemia	18/50 (36%)	30/50 (60%) p = 0.014	29/50 (58%) p = 0.022

[a]p-values were calculated using the Fisher Exact Test.

The diagnoses of mononuclear cell leukemia were classified as stage 1 (early), stage 2 (intermediate), or stage 3 (advanced and probably fatal).

The following criteria were used for assigning these stages:

Stage 1 -- Spleen not enlarged or only moderately enlarged, with few suspicious cells surrounding Malpighian corpuscles of the spleen, and only very few neoplastic cells in the blood vessels of the lung.

Stage 2 -- More severe than in the first category; spleen and/or liver only moderately enlarged, with neoplastic cells in the liver sinusoids and in the blood vessels of other organs.

Stage 3 -- Enlarged spleen and liver, with many neoplastic cells in the liver sinusoids and in the blood vessels of other organs.

Table 3-3 summarizes the distribution of these three stages of mononuclear cell leukemia in the male and female rats studied.

The strength of the evidence for carcinogenicity presented here for F344 rats hinges on the resolution of issues raised regarding the uncertainty in the assignment of incidence within stages of mononuclear cell leukemia in the treated F344 rats and the high and variable incidence of mononuclear cell leukemia in F344 untreated rats, particularly males.

Table 3-4 presents an analysis of adenomas and carcinomas in the rats exposed to PCE. Tubular cell adenomas or carcinomas were observed only in male rats, but with a dose-related incidence that is not statistically significant. Renal tubular adenomas were: in control, 1/49; 200 ppm, 3/49; 400 ppm, 2/50. Carcinomas were: in control, 0/49; 200 ppm, 0/49; 400 ppm, 2/50. The historical incidences of renal tubular adenomas in the NTP studies were 4/1720, or less than 0.2% ± 0.7%; no malignant tubular cell carcinomas have been observed historically. In addition, dose-related increased incidences of renal tubular karyomegaly were observed in both male and female rats. Tubular cell hyper-

TABLE 3-3. CLASSIFICATION OF MONONUCLEAR CELL LEUKEMIA IN RATS EXPOSED TO PCE BY INHALATION

Group	Number of rats with mononuclear cell leukemia	Stage		
		1	2	3
Males				
Control	28	5	3	20
200 ppm	37	6	7	24
400 ppm	37	4	6	27
Females				
Control	18	3	5	10
200 ppm	30	6	6	18
400 ppm	29	2	6	21

SOURCE: NTP, 1985.

TABLE 3-4. TUBULAR CELL ADENOMAS AND CARCINOMAS OF THE KIDNEY IN RATS EXPOSED TO PCE BY INHALATION[a]

Group	Control	200 ppm[a]	400 ppm[a]
Males			
Tubular cell adenomas	1/49 (2%)	3/49 (6%)	2/50 (4%)
Tubular cell adenocarcinomas	0/49 (0%)	0/49 (0%)	2/50 (4%)
Tubular cell adenomas or adenocarcinomas	1/49 (2%)	3/49 (6%) p = 0.259[b]	4/50 (8%) p = 0.070[b]
Females			
Tubular cell adenomas	0/50 (0%)	0/49 (0%)	0/50 (0%)
Tubular cell adenocarcinomas	0/50 (0%)	0/50 (0%)	0/50 (0%)
Tubular cell adenomas or adenocarcinomas	0/50 (0%)	0/49 (0%)	0/50 (0%)

[a]Historical incidence at the Battelle Pacific Northwest Laboratories: 0/100; historical incidence in NTP studies: 4/1,720, 0.2% ± 0.7%; no malignant tubular cell tumors were observed.

[b]p-values were calculated for tubular cell adenomas or carcinomas using the Fisher Exact Test.

SOURCE: NTP, 1985.

plasias were also observed; these may be preneoplastic lesions.

In the rats, there were no statistically significant increased incidences of tumors in other organs examined. In particular, no neoplastic changes were observed in the respiratory tracts of either male or female rats. However, there was an increased incidence of squamous cell metaplasia in the nasal cavities of the exposed male rats (0/50, control; 5/50, 200 ppm; 5/50, 400 ppm).

3.3. RESULTS OF THE MOUSE STUDY

Figure 3-3 presents the growth curves for male and female mice exposed to PCE by inhalation for 2 years. As indicated in the figure, there was no significant change in the mean body weights of dosed animals as compared with controls. The numbers of mice surviving the 2-year inhalation study of PCE are shown in Table 3-5 and Figure 3-4. The survival of male mice was significantly reduced at both 100 and 200 ppm PCE exposures, as compared with controls: low dose, 25/50 (50%); high dose, 32/50 (64%); control, 46/50 (96%). In female mice, survival was reduced at 200 ppm as compared with controls: low dose, 31/50 (62%); high dose, 17/50 (34%); control, 36/50 (72%). Survival of male control mice was quite high. The high early mortality observed in female mice [31/50 (60%)] may be attributed to the high incidence of hepatocellular carcinomas.

The incidences of liver tumors in the male and female B6C3F1 mice exposed to PCE are summarized in Table 3-6. In the male mice, PCE induced statistically significant increased incidences of hepatocellular carcinomas at both the 100 and 200 ppm exposure levels, as compared with controls. These tumor incidences were: low dose, 25/49 (51%) ($p < 0.001$); high dose, 26/50 (52%) ($p < 0.001$); control, 7/49 (14%). Hepatocellular adenomas alone were not statistically significant at either the 100 ppm or the 200 ppm exposure concentration. The combined incidences of hepatocellular adenomas and carcinomas were sta-

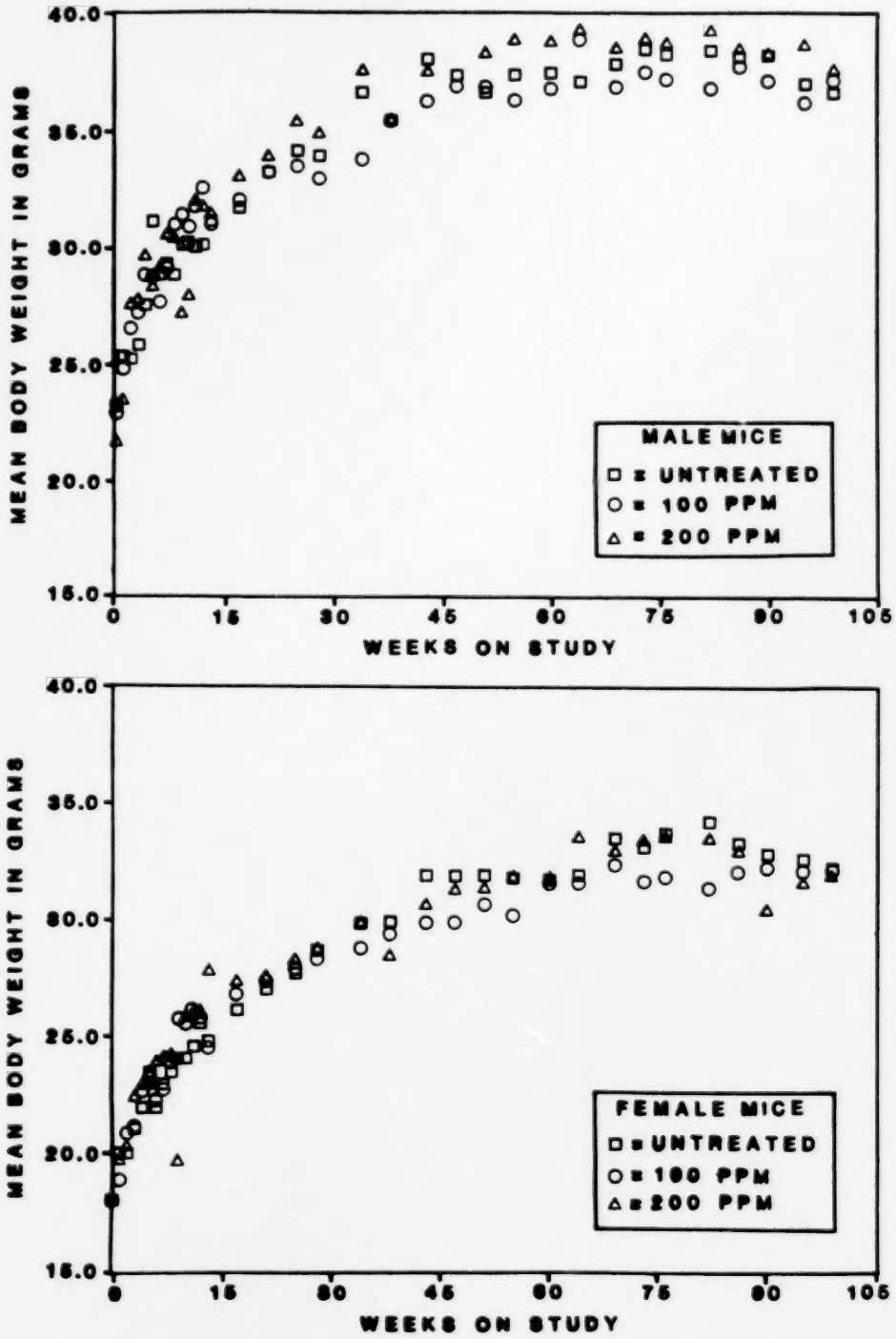

Figure 3-3. Growth curves for mice exposed to PCE by inhalation for 2 years.

SOURCE: NTP, 1985.

TABLE 3-5. SURVIVAL OF MICE
IN THE NTP 2-YEAR INHALATION BIOASSAY OF PCE

Group	Control	100 ppm	200 ppm
Males			
Animals initially in study	50	50	50
Nonaccidental deaths	3 (6%)	25 (50%)	18 (36%)
Animals missexed	1 (2%)	0 (0%)	0 (0%)
Killed at termination	46 (92%)	25 (50%)	32 (64%)
Females			
Animals initially in study	50	50	50
Nonaccidental deaths	11 (22%)	17 (34%)	30 (60%)
Accidentally killed	2 (4%)	2 (4%)	1 (2%)
Animals missexed	1 (2%)	0 (0%)	0 (0%)
Killed at termination	36 (72%)	31 (62%)	17 (34%)[a]

[a]Two mice died during termination period.

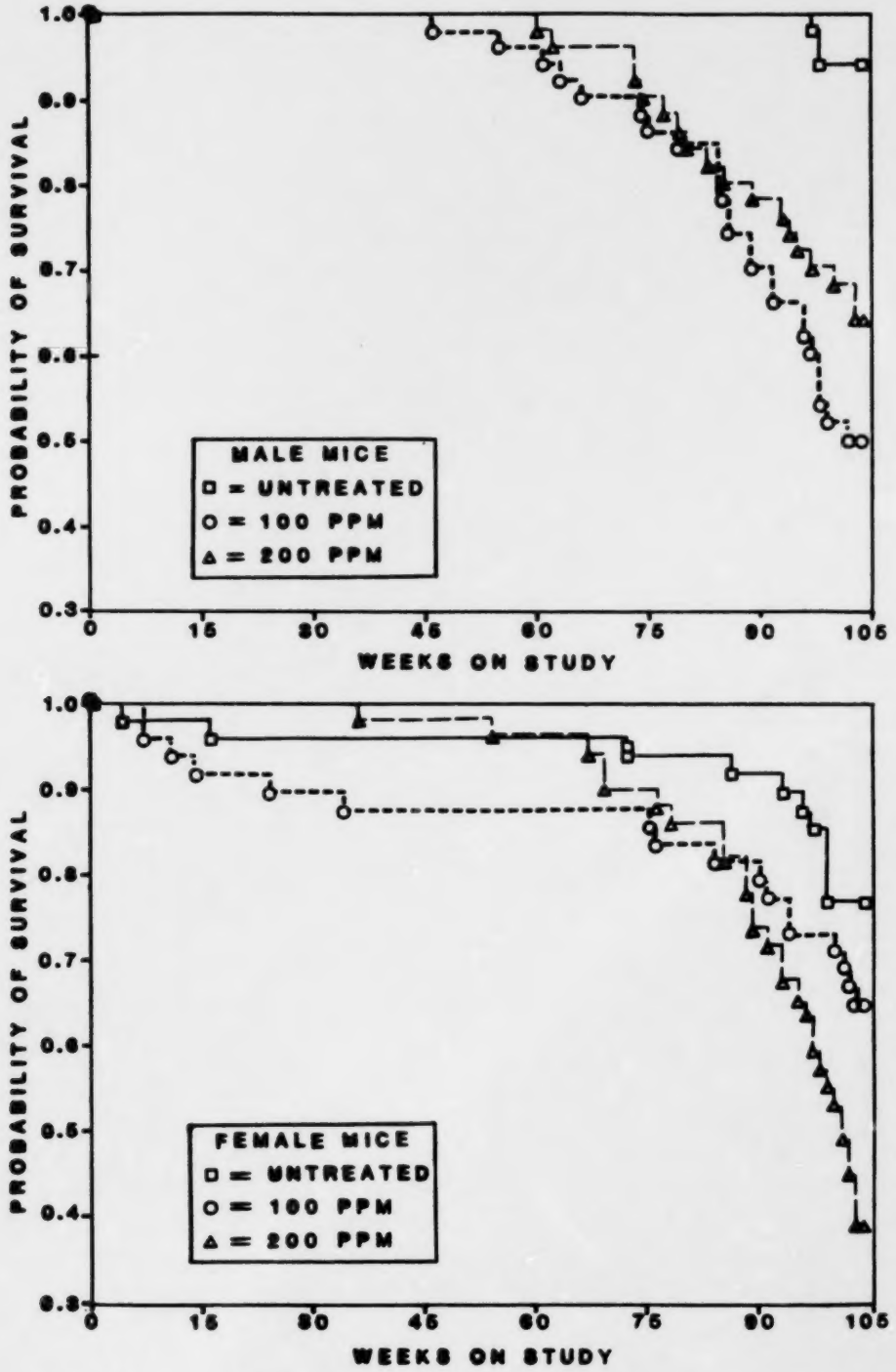

Figure 3-4. Kaplan-Meier survival curves for mice exposed to PCE by inhalation for 2 years.

SOURCE: NTP, 1985.

TABLE 3-6. LIVER TUMORS IN MICE EXPOSED TO PCE BY INHALATION

Group	Control[a]	100 ppm[a,b]	200 ppm[a,b]
Males			
Hepatocellular adenoma	11/49 (22%)	8/49 (16%) N.S.	18/50 (36%) N.S.
Hepatocellular carcinoma	7/49 (14%)	25/49 (51%) p<0.001	26/50 (52%) p<0.001
Hepatocellular adenoma or carcinoma	16/49 (33%)[c]	31/49 (63%)[d] p=0.002	40/50 (80%)[e] p<0.001
Females			
Hepatocellular adenoma	3/48 (6%)	6/50 (12%) N.S.	2/50 (4%) N.S.
Hepatocellular carcinoma	1/48 (2%)	13/50 (26%) p<0.001	36/50 (72%) p<0.001
Hepatocellular adenoma or carcinoma	4/48 (8%)	17/50 (34%)[f] p=0.001	38/50 (76%) p<0.001

[a]The denominators represent the numbers of animals examined histologically.
[b]p-values were calculated using the Fisher Exact Test.
[c]Controls, male - 2 mice had both adenoma and carcinoma.
[d]Low dose, male - 2 mice had both adenoma and carcinoma.
[e]High dose, male - 4 mice had both adenoma and carcinoma.
[f]Low dose, female - 2 mice had both adenoma and carcinoma.
N.S. = not significant.

SOURCE: NTP, 1985.

tistically significant as compared with the controls. The incidences were: low dose, 31/49 (63%) ($p = 0.002$); high dose, 40/50 (80%) ($p < 0.001$); control, 16/49 (33%).

In female mice, PCE induced a statistically significant increased incidence of hepatocellular carcinomas at the 100 and 200 ppm exposure concentrations, as compared with the controls. These incidences were: low dose, 13/50 (26%) ($p =< 0.001$); high dose, 36/50 (72%) ($p < 0.001$); control, 1/48 (8%). Incidences of adenomas were not statistically significant. However, the combined incidences of adenomas and carcinomas were statistically significant as compared with controls. The incidences were: low dose, 17/50 (34%) ($p = 0.001$); high dose, 38/50 (76%) ($p < 0.001$); control, 4/48 (8%). Carcinogenic effects were not seen in any of the other organ sites, including the respiratory system. In addition, dose-related increases in liver degeneration, hepatocellular necrosis, and hepatocellular nuclear inclusion were observed in both male and female mice exposed to PCE. Dose-related renal tubular cell karyomegaly was observed in the kidneys of both male and female mice, but no dose-related tubular cell carcinomas were found in the mice.

The NTP mouse inhalation bioassay further confirms the earlier National Cancer Institute study in mice (NCI, 1977) in which PCE induced a statistically significant increased incidence of hepatocellular carcinomas by gavage.

The NTP (1985, draft) study was audited by Dynamac Corporation for completeness, consistency, accuracy, and for procedures consistent with good laboratory practice requirements. The audit revealed no major problem in the conduct of the study or in the collection and documentation of the experimental data.

3.4. SUMMARY

In the NTP (1985, draft) inhalation bioassay, groups of 50 male and 50 female F344/N rats and B6C3F1 mice were exposed to PCE (99.9% pure), 6 hours/

day, 5 days/week, for 2 years. The exposure concentrations used were 0, 200, and 400 ppm for rats and 0, 100, and 200 ppm for mice. At the 200 and 400 ppm exposure concentrations, PCE induced a marginally statistically significant increase of mononuclear cell leukemia in male rats. PCE also induced an increased incidence of renal tubular cell adenomas or carcinomas (combined) in male rats. This incidence shows a dose-related trend, although it is not statistically significant.

In female rats at the 200 and 400 ppm exposure concentrations, a statistically significant increased incidence of mononuclear cell leukemia was observed. In male and female B6C3F1 mice, PCE induced a statistically significant increased incidence of hepatocellular carcinomas and/or adenomas at both the 100 and the 200 ppm exposure concentrations.

4. QUANTITATIVE RISK ASSESSMENT

This section updates the inhalation unit risk estimate for PCE reported in the July 1985 Health Assessment Document for Tetrachloroethylene by using data from the NTP (1985) inhalation bioassay, which is the first lifetime inhalation study to show evidence of the carcinogenicity of PCE in mice and rats. These data provide a basis for directly estimating the risk of PCE by the inhalation route, and an opportunity to test the reasonableness of the July 1985 risk estimate, which was developed using the gavage data from the NCI bioassay (1977). The inhalation unit risk estimate for PCE based on tumor incidence data from a gavage study in mice and the assimilated dose metabolized, was accomplished by utilizing metabolism information for both animals and humans, expressing dose as the amount of PCE metabolized. On this basis the reported inhalation unit risk is $4.8 \times 10^{-7}/\mu g/m^3$. This estimate was found to be comparable to three other estimates using different data bases and/or different methods of calculation. The details of these calculations are presented in the Health Assessment Document (U.S. EPA, 1985, pp. 9-53 to 9-61).

The new unit risk estimate for inhalation also utilizes metabolized dose-tumor incidence relationships derived from exposure dose-metabolism relationship data in both animal and human experiments. The uncertainty associated with extrapolating from animals to humans is reduced by the use of actual human exposure dose-metabolism relationship estimates, using urinary trichloroacetic acid as a measure of metabolism, in addition to the use of comparable animal data.

For comparison, we have also used a physiologically-based pharmacokinetic model to predict the amount of PCE metabolized in the NTP bioassay animals.

4.1. DATA AVAILABLE FOR RISK CALCULATION

The NTP (1985) inhalation bioassay shows evidence of the carcinogenicity

of PCE in rats (mononuclear cell leukemia) and mice (hepatocellular adenomas and carcinomas). The assays for both species have been selected for estimation of carcinogenic risk to humans for inhalation exposure.

4.1.1. NTP Rat Inhalation Assay

Long-term (103 weeks) carcinogenicity studies were conducted by inhalation exposure (0, 200, and 400 ppm, 6 hours/day, 5 days/week) on groups of 50 male and 50 female Fischer 344/N rats. The animals were placed on study at 8 to 9 weeks of age and killed at 112 to 113 weeks of age.

Table 3-2 shows the overall incidence rates for mononuclear cell leukemia. Although the survival of male rats in the high-dose group is significantly reduced when compared to the control group, the difference appears to be significant only at the late stage (after 80 weeks) of the experiment. Because the survivalship differs only at the late stage of the experiment and a large proportion of leukemias were found at the termination of the bioassay, it is more appropriate to use the incidence rate data, rather than the time-to-death data, to calculate the lifetime cancer risk.

4.1.2. NTP Mouse Inhalation Assay

Lifetime (103 weeks) carcinogenicity studies were conducted by inhalation exposure (0, 100, and 200 ppm, 6 hours/day, 5 days/week) on groups of 50 male and 50 female B6C3F1 mice. The animals were placed on study at 8 to 9 weeks and killed at 112 to 113 weeks. Exposure to PCE at 100 or 200 ppm reduced survival of male mice, and at 200 ppm reduced survival of female mice. Most of the deaths in treated animals occurred after week 82, and there is evidence that the unscheduled deaths may have been influenced by the high incidence of hepatocellular carcinomas. However, for the same reasons as discussed for rats, the incidence data are considered more appropriate for risk calculation than the time-to-death data. Table 3-4 shows the overall incidence rates for

hepatocellular adenomas and carcinomas, which, combined, show a dose-related incidence of hepatocellular neoplasms in both male and female mice.

4.2. POSSIBLE MECHANISMS LEADING TO A CARCINOGENIC RESPONSE FOR PCE

Possible mechanisms proposed for carcinogenesis include direct interaction of a chemical or its metabolites with DNA, long-term tissue injury, stimulation of cell proliferation, immunosuppression, hormonal imbalances, or release of altered cells from growth control (Weisburger and Williams, 1980, 1981). The mechanism(s) by which PCE causes cancer is not known and therefore a matter of some speculation. The current knowledge of PCE metabolism and the acute cellular toxicity directly related to reactive metabolites suggest different cellular processes that may possibly lead to carcinogenic activity. One process may involve cell death induced by the cellular toxicity of PCE followed by the consequent stimulation of DNA replication associated with cell multiplication, resulting in an indirect or promoting effect. The continuing process of DNA replication results in increased proportions of single-strand DNA, which is more susceptible to irreversible binding by reactive intermediates than is double-strand DNA. Alternatively, it is possible that PCE acts by stressing the fidelity of replication, thus increasing the possibility of introducing DNA transcript errors. A general process proposing that necrosis constututes a necessary precursor for PCE liver carcinogenicity is consistent with a threshold effect at the necrotizing doses at which all death and turnover occur. It is also consistent with the negative or inconclusive results of the majority of mutagenicity assays.

Peroxisome proliferation has been linked with cancer in rodents (Moody and Reddy, 1978; Reddy et al., 1980; Prout et al., 1985; Green and Prout, 1985). Trichloroacetic acid (TCA) resulting from the biotransformation of PCE has been shown to induce liver peroxisome proliferation in mice but not in rats (Green,

1986). It has been hypothesized that the species difference in the hepatocarcinogenicity of PCE seen between rats and mice is possibly due to a species difference in peroxisome proliferation resulting from higher TCA blood levels in the mice (Green, 1986).

Increased reactive oxygen species formed by the peroxisomes may result in DNA damage, or the peroxisome proliferation may result in formation of the low amounts of DNA alkylation that have been observed following exposure to PCE. However, a cause-and-effect relationship between liver peroxisome proliferation and PCE hepatocarcinogenicity in mice has not been established. This mechanism does not account for the mononuclear cell leukemias and renal tubular neoplasms seen in rats.

Another general process that may lead to carcinogenicity involves the metabolic production of active PCE metabolites (tetrachloroethylene oxide) and their direct interaction with DNA. This genotoxic process could be expected to be modulated by various homeostatic mechanisms, such as DNA repair and immunological surveillance, but it is generally regarded as lacking a threshold. This mechanism is supported by the mutagenicity of the PCE-epoxide and the fact that DNA binding of PCE metabolites has been observed, even though only at low doses.

In the absence of definitive evidence solely supporting any specific one of the likely processes operative in the carcinogenic activity of PCE, the risk assessment performed by the Carcinogenic Assessment Group considers the process which is generally accepted--the genotoxic mechanism--to be associated with the greatest risk. The risk of PCE carcinogenicity by this process, with its lack of threshold, is appropriately estimated by a mathematical model predicting zero incremental risk only at zero exposure. In addition, at the present time, the lack of sufficient understanding about the mechanisms of action involved

in carcinogenesis does not allow for a distinction between chemicals acting directly with DNA and those which do not, nor can those which have not been shown to be genotoxic be considered to have identifiable population thresholds or to be safer than those which are considered to be genotoxic (Pereira, 1984). More research is required on the mechanism(s) of action of PCE before a threshold model would be appropriate for carcinogen risk assessment (Pereira, 1984).

4.3. RELEVANCE OF METABOLISM TO QUANTITATIVE RISK ASSESSMENT

The general considerations involved in the scaling of toxicologic effects, including carcinogenicity, among species (including man) have been outlined for PCE in the Health Assessment Document for Tetrachloroethylene (U.S. EPA, 1985), which also presented information on the kinetics, metabolism, and covalent binding properties of PCE in rat and mouse species and metabolism and kinetics in humans. This information may be summarized as follows:

(1) There is evidence that the liver and kidney toxicity and the carcinogenic potential of PCE and other halogenated ethylenes are dependent on the metabolic conversion of these compounds to reactive epoxides and other biologically reactive intermediates of metabolism (Figure 4-1) (Bolt et al., 1982; U.S. EPA, 1985, Chap. 5).

(2) The reactive metabolites of PCE bind irreversibly to cellular macromolecules *in vitro* and *in vivo* (Costa and Ivanetich, 1980; Pegg et al., 1979; Schumann et al., 1980). Significant binding to hepatic DNA has not been demonstrated to occur *in vivo* (Schumann et al., 1980). Irreversible binding to other cellular macromolecules (proteins and lipids) *in vivo* occurs independently of route of administration (gavage or inhalation) but proportionally to the amount metabolized (Pegg et al., 1979; Schumann et al., 1980). Indices of hepatocellular toxicity and cellular damage have been shown to correlate

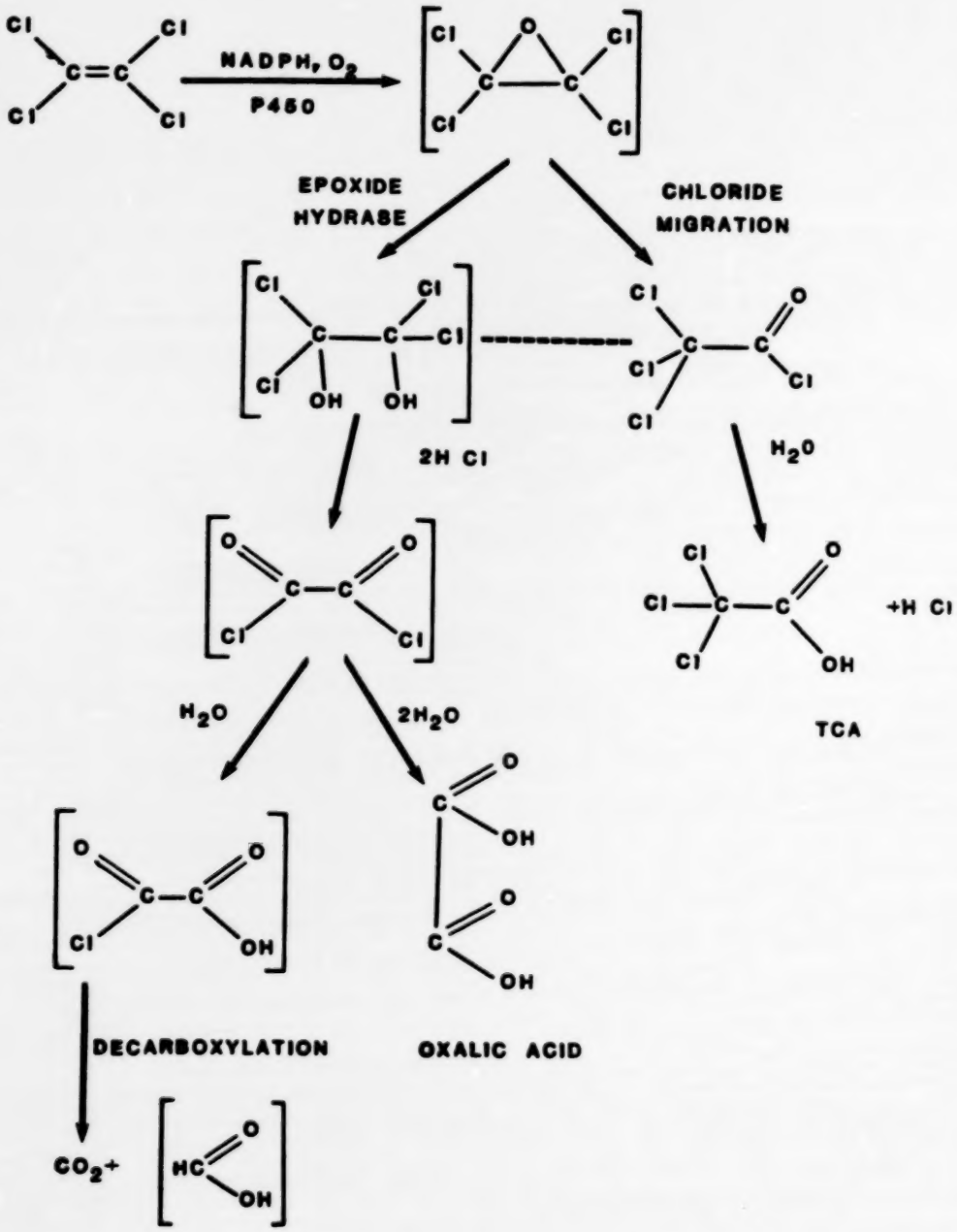

Figure 4-1. Postulated scheme for the metabolism of PCE.

SOURCE: U.S. EPA, 1985.

linearly with metabolism (Buben and O'Flaherty, 1985).

(3) The metabolism and kinetics of PCE have been studied in three species: mice, rats, and humans. In each of these species there is evidence that metabolism of PCE after oral (mice, rats) or inhalation exposure (mice, rats, humans) is rate-limited and proceeds according to Michaelis-Menten kinetics (Filser and Bolt, 1979; Pegg et al., 1979; Schumann et al., 1980; Buben and O'Flaherty, 1985; Ohtsuji et al., 1983; U.S. EPA, 1985, Chap. 5). There is no comparative experimental evidence that the metabolic pathway for PCE qualitatively differs for the above-mentioned species. The metabolic capacity for the metabolism of PCE by mice and rats has been found to be proportional to body surface area for these two species (U.S. EPA, 1985, Chap. 9).

(4) Elimination of PCE following inhalation exposure in humans is triphasic, with a terminal first-order elimination half-time of 55 to 65 hours; for the rat, the dominant half-time of elimination is 7 hours after inhalation or oral exposure. These long half-times indicate that with chronic daily exposures in humans or animals (conditions of carcinogenicity bioassays) complete elimination (by metabolic and pulmonary routes) would not occur within the 24-hour dosing interval and that body accumulation would occur until a plateau of equilibrium is reached between body concentration and inhalation or oral exposure dose.

4.4. INHALATION EXPOSURE IN HUMANS

PCE is readily absorbed through the lungs into the blood by first-order passive diffusion processes. Two major processes account for the known elimination of PCE from the body: 1) pulmonary excretion of unchanged PCE, and 2) metabolism of PCE to urinary metabolites. The metabolism of PCE appears to be very limited in humans, as it is in experimental animals. Only a small percentage of the estimated amounts absorbed by inhalation are metabolized to

trichloroacetic acid (TCA) and other chlorinated metabolites found in the urine (U.S. EPA, 1985, Chap. 5). Saturation of metabolism has been estimated to occur in humans at relatively low exposure concentrations (between 100 and 400 ppm PCE in inspired air). Estimates of the extent of PCE metabolism in humans have been made from balance studies by measuring urinary metabolites after accounting for a retained inhalation exposure dose (U.S. EPA, 1985, Chap. 5). However, there are several difficulties associated with balance studies in humans. For example, problems are encountered in obtaining an accurate measurement of the retained dose of PCE from inhalation exposure. In addition, the available balance studies in humans are incomplete. One shortcoming in some of the older PCE studies has been the imprecision of the analytical methodologies used; for example, the Fujiwara reaction for measuring metabolites. Also, urinary sampling may not have been sufficient considering the long half-life of PCE metabolites. In addition, individual variations among subjects can be relatively high. Only urinary TCA was used in these studies as a measure of metabolism, and the possibility exists that significant amounts of metabolites other than TCA are excreted in the urine or by other routes. It has been proposed that a much higher percentage of the dose is actually metabolized, and that a so-far unrecognized pathway may exist for PCE and for other metabolites which have not yet been identified. However, measuring the amount of urinary TCA is one approach that may be used to compare the metabolized dose of PCE among species. The metabolized dose may be considered to be the effective dose in organ toxicity and carcinogenicity.

4.5. CALCULATION OF METABOLIZED DOSE FROM INHALATION STUDIES

4.5.1. Direct Estimation from Metabolism Experiments

4.5.1.1. Rat--Pegg et al. (1979) and Schumann et al. (1980) determined body burdens of Sprague-Dawley rats and B6C3F1 mice after 6-hour inhalation expo-

sures. The animals were exposed to ^{14}C-PCE and the amount metabolized as a fraction of the body burden was estimated from radioactivity in urine, feces, expired air, etc., and unchanged ^{14}C-PCE in exhaled air. Their results for exposed rats are given in Table 4-1.

Total recovery of ^{14}C-radioactivity from 6-hour inhalation of 10 ppm and 600 ppm ^{14}C-PCE in rats was nearly directly proportional to exposure concentration. However, the absolute amount metabolized (10 ppm, 0.47 mg-equivalents; 600 ppm, 9.11 mg-equivalents PCE) was not proportional to exposure concentration and decreased both in absolute proportions and as a percentage of the total assimilated dose (10 ppm, 32%; 600 ppm, 12%). Thus, these data from rats confirm in this species limited and saturable metabolism of PCE consistent with dose-dependent Michaelis-Menten kinetics, as has been indicated by kinetic inhalation studies of Filser and Bolt (1979). These data are also in accord with the demonstration by Buben and O'Flaherty (1985) of dose-dependent Michaelis-Menten kinetics for PCE in mice.

The relationship between the rate of PCE metabolism in adult male Sprague-Dawley rats (or the amount of PCE metabolized per unit body weight per 6-hour exposure, M) and exposure concentration dose, d (ppm), can be expressed by a Michaelis-Menten-like equation

$$M = V \times d/(K + d)$$

where V and K are constants to be estimated from the data. In order to utilize the human data (metabolites in urine) for interspecies extrapolation, the amount of urinary metabolites in animals is used to establish the dose-metabolism relationship. Using the data of urinary metabolites for rats and assuming Fischer 344 rats metabolize PCE qualitatively similar to Sprague-Dawley rats with inha-

TABLE 4-1. RECOVERY OF ^{14}C-PCE RADIOACTIVITY AFTER INHALATION EXPOSURE FOR 6 HOURS TO SPRAGUE-DAWLEY RATS AND B6C3F1 MICE[a]

	Rats (av. of 3)[a]		Mice (av. of 3)
	10 ppm (mg-equivalents PCE[b])	600 ppm	10 ppm
Expired unchanged	1.008 (68%)[c]	68.39 (88%)[c]	0.048 (12%)[c]
Metabolized			
$^{14}CO_2$	0.053	0.54	0.032
Urine	0.275	4.54	0.285
Feces	0.076	2.36	0.027
Carcass	0.063	1.67	0.012
	0.467 (32%)[c]	9.11 (12%)[c]	0.356 (88%)[c]
Total	1.475	77.50	0.404

[a]Average experimental animal weights (grams): 250, rat; 24.5, mouse. The animals were exposed to 10 or 600 ppm ^{14}C-PCE for 6 hours and maintained in metabolism cages for 72 hours after exposure.
[b]Each value represents the mean of data from three animals.
[c]Numbers in parenthesis indicate the fraction of total administered dose.

SOURCES: Adapted from Pegg et al., 1979 and Schumann et al., 1980.

lation exposure (Table 4-1), the dose-metabolism relationship is determined to be

$$M\ (mg/kg) = V \times d\ (ppm)/(K + d)$$

with V and K estimated to be 24.64 mg/kg and 213.96 ppm, respectively. This relationship is used to estimate the amount of PCE metabolized for a 6-hour exposure at 200 or 400 ppm in the NTP (1985) rat bioassay. The amounts metabolized (urinary excretion) are 11.90 mg/kg/day and 16.05 mg/kg/day for 6-hour exposures, corresponding, respectively, to 200 and 400 ppm ambient air concentrations. It may be noted that doubling the daily inhalation exposure concentration from 200 to 400 ppm results in only a 30% increase in the amount of PCE metabolized. The two doses represent approximately 48% and 65% of total metabolic capacity. These observations may, in part, explain the failure to observe an exposure dose-leukemia response relationship for this rat bioassay.

4.5.1.2. Mouse--The dose-dependent Michaelis-Menten kinetics for the metabolism of PCE by mice after oral administration have been amply demonstrated by Buben and O'Flaherty (1985). For inhalation exposure, however, few data are available pertinent to the conditions of the NTP (1985) bioassay, from which to derive a comparative relationship between exposure concentration and metabolism, as was done in the Buben and O'Flaherty (1985) oral study.

Watanabe and associates (Pegg et al., 1979; Schumann et al., 1980) assessed the amount of PCE metabolism accrued from a 10 ppm, 6-hour exposure to B6C3F1 mice (Table 4-1). The metabolism of PCE in mice was not determined at other exposure concentrations. The exposure levels of the NTP inhalation bioassay in mice were 10- and 20-fold higher. At the 10 ppm exposure level, the absolute amount of metabolites in urinary excretion is comparable between the

two species; however, expressed in mg/kg, the amount of PCE metabolites is about 10 times greater for mice than for rats (11.6 mg/kg vs. 1.1 mg/kg). This ratio should not be expected to hold at higher concentrations because at higher concentrations the metabolic rate is dominated by V_{max} in the Michaelis-Menten equation. Theoretically, the ratio of V_{max} between two species equals unity if V_{max} is expressed in terms of metabolic rate as represented by $mg/W^{2/3}$, or, if V_{max} is expressed in terms of mg/kg, is equal to $(W_1/W_2)^{1/3}$ where W's are body weights for animals of each species. Therefore, at high doses one might expect to see that mice metabolize about twice the amount of PCE, in terms of mg/kg, as do rats [i.e., $(0.25/0.0245)^{1/3} = 2.2$]. This ratio is supported by the observation from oral studies (Table 4-2) that at 500 mg/kg, the ratio of the absolute amount of urinary PCE metabolites between mice and rats is 0.267, or 2.7 on a mg/kg basis [i.e., $(1.53/0.0245)/(5.72/0.25) = 2.7$]. The amount metabolized of a 500 mg/kg oral dose in rats is approximately equal to the amount metabolized of a 600 ppm inhalation exposure in this species. To calculate the metabolized dose for the NTP mice, it is assumed that mice metabolize approximately 5 times (in terms of mg/kg) more than rats at the cancer bioassay doses of 100 and 200 ppm on the basis that mice metabolize greater amounts (in terms of mg/kg) than rats, ranging from 10 times greater at 10 ppm to 2 to 3 times greater at higher doses. Therefore, the metabolized doses of PCE for mice, based on urinary metabolites, corresponding to 100 and 200 ppm 6-hour exposures are, respectively, 39.24 mg/kg/day and 59.50 mg/kg/day--or approximately five times the values calculated from the dose-metabolism relationship for rats, when expressed in terms of mg/kg.

4.5.2. Metabolized Dose Predicted by a PB-PK Model

In addition to the (urinary) metabolized doses that are calculated directly from the empirical data and are presented in the previous sections, we have

TABLE 4-2. DISPOSITION OF ^{14}C-PCE RADIOACTIVITY FOR 72 HOURS AFTER SINGLE ORAL DOSES TO SPRAGUE-DAWLEY RATS AND B6C3F1 MICE

	Rats (av. of 3)[a]		Mice (av. of 3)
	1 mg/kg (0.25 mg/animal) mg-equivalents PCE per animal	500 mg/kg (125 mg/animal) mg-equivalents PCE per animal	500 mg/kg (12.25 mg/animal) mg-equivalents PCE per animal
Expired unchanged	0.174 (71%)	110.67 (90%)	8.90 (83%)
Metabolized			
$^{14}CO_2$	0.007	0.57	0.14
Urine	0.040	5.72	1.53
Feces	0.015	4.82	0.13
Carcass	0.008	1.41	0.05
	0.070 (29%)	12.52 (10%)	1.85 (17%)
Total	0.244	123.19	10.75

[a]Based on average experimental animal weight (grams): 250, rat; 24.5, mouse.

SOURCES: Adapted from Pegg et al., 1979 and Schumann et al., 1980.

also calculated the amount metabolized by using the physiologically-based pharmacokinetic (PB-PK) model. An explanation of the construction of the model is given in Appendix A. The PB-PK model predicts only the total amount metabolized, not the urinary metabolites as previously calculated. The metabolized doses predicted by the PB-PK model are comparable to those calculated directly from the empirical data (Table 4-3), considering the fact that urinary metabolites account for about 50% to 80% of the total metabolites and the fact that the metabolized doses for mice are slightly underestimated (approximately three times), by the PB-PK model, as discussed in section A.3.2.2. of Appendix A.

4.6. HUMAN METABOLIZED DOSE AT LOW-LEVEL AMBIENT EXPOSURE

Estimation of the extent of metabolism in humans have been made from several balance studies by accounting for a retained dose after inhalation exposure through measuring metabolites excreted in the urine. The difficulties associated with these balance studies in humans have already been mentioned in section 4.3. However, urinary TCA measurements can reasonably be used to make species comparisons of metabolized dose. The data in humans are represented here by studies selected on the basis of the lower exposure levels more commensurate with anticipated environmental concentrations.

A study by Bolanowska and Golacka (1972) provides some information on the relationship between the air concentration of PCE and the amount metabolized in urinary excretion. In this study, five subjects were exposed to 390,000 $\mu g/m^3$ of PCE (approximately 50 ppm) for 6 hours. The excretion rate of metabolites in urine (mg/hr) is presented graphically over the 20-hour period during and after exposure. The total amount of metabolites in urine (area under curve) is estimated to be about 13 mg. The area under the curve from 20 hours to infinity is calculated by $C \times t_{1/2}/0.693$, where C is the concentration at the last sampling time (i.e., 20 hours) and $t_{1/2}$, the half-life, is assumed to be

TABLE 4-3. COMPARATIVE ESTIMATES OF METABOLIZED DOSES IN THE NTP (1985) INHALATION BIOASSAY

Species	Exposure concentration (ppm)	Direct estimate of urinary metabolites (mg/kg)	Total metabolites estimated by PB-PK model (mg/kg)[a]
Rats	200	11.90	19.39
	400	16.05	28.57
Mice	100	39.24	19.77[b]
	200	59.50	30.00[b]

[a]The PB-PK model can predict only the total amount of metabolites. The values are total metabolized dose over 72 hours after a single 6-hour exposure. These values are presented here for the purpose of comparison only; they are not used for risk calculation.

[b]The metabolized doses for mice are slightly underestimated (approximately 3 times) due to the use of under-valued physiological parameters, in particular the alveolar ventilation rate (see section A.3.2.2. in Appendix A).

100 hours. Under the assumption that the amount metabolized is linearly proportional to the air concentration below this concentration, the amount metabolized (in urine) associated with 1 $\mu g/m^3$ of PCE in air is 13 mg/390,000 = 3.33×10^{-5} mg for a 6-hour exposure.

Another study that supports the above estimate is that of Fernandez et al. (1976) in which humans were exposed to PCE vapor at 150 ppm for 8 hours. The quantity of PCE metabolized and eliminated in urine over 72 hours was about 25 mg. This is clearly less than the total amount metabolized from the 8-hour exposure because of the long half-life in the final phase of urine elimination. As an approximation, the (urinary) metabolized dose associated with 1 $\mu g/m^3$ of PCE in air is calculated to be

$$1\ \mu g/m^3 \times (25\ mg/150\ ppm) \times (6\ hr/8\ hr)/6908/\mu g/m^3/ppm = 1.8 \times 10^{-5}\ mg$$

for a 6-hour exposure, given the fact that 1 ppm = 6908 $\mu g/m^3$. This value is less than the value of 3.33×10^{-5} mg calculated on the basis of Bolanowska and Golacka (1972). The value 3.33×10^{-5} mg/6 hours will be used for risk calculation because it is obtained from a lower concentration (50 ppm), where the assumption that the dose-metabolism relationship is linear is not unreasonable.

4.7. CALCULATION OF UNIT RISK

4.7.1. Based on the Metabolized Dose Estimated Directly from the Empirical Data

In this section, the incremental lifetime cancer risk over background due to 1 $\mu g/m^3$ of PCE in ambient air (i.e., unit risk) is calculated. The calculation procedure, the interpretation, and the appropriate use of the concept of unit risk were presented in the Health Assessment Document for PCE (U.S. EPA, 1985). The data used for the risk calculation are given in Tables 4-4 and 4-5.

TABLE 4-4. NTP METABOLIZED DOSE
AND INCIDENCE OF MONONUCLEAR CELL LEUKEMIA
IN FISCHER 344 RATS

Group	Exposure concentration (ppm)	Human equivalent metabolized dose ($mg/W^{2/3}/day$)[a]	Incidence rate
Males	0	0	28/50
	200	6.26	37/50
	400	8.45	37/50
Females	0	0	18/50
	200	5.81	30/50
	400	7.84	29/50

[a]Metabolized dose = M(mg/kg/day) x (5/7) x $W^{1/3}$, where M(mg/kg/day) is the direct estimate of the dose metabolized taken from Table 4-3; the factor 5/7 reflects that animals were exposed only 5 days/week; and W = 0.40 kg for male rats and 0.32 kg for female rats. For convenience, the notation $mg/W^{2/3}$ is used to indicate the amount metabolized in terms of mg per two-thirds of body weight, which is assumed to be proportional to the body surface area.

TABLE 4-5. NTP METABOLIZED DOSE
AND INCIDENCE OF LIVER TUMORS IN MICE

Group	Exposure concentration (ppm)	Human equivalent metabolized dose ($mg/W^{2/3}/day$)[a]	Incidence rate	
			Carcinomas	Adenomas/carcinomas
Males				
	0	0	7/49[b]	16/49
	100	9.37	25/47	31/47
	200	14.21	26/50	40/50
Females				
	0	0	1/46	4/46
	100	8.92	13/42	17/42
	200	13.52	36/47	38/47

[a]Metabolized dose = M(mg/kg/day) x (5/7) x $W^{1/3}$, where M(mg/kg/day) is the direct estimate of metabolized dose taken from Table 4-3; 5/7 is a factor to adjust for 5 days exposure in a week; and W = 0.0374 kg for male mice and 0.0322 kg for female mice.
[b]Only animals surviving beyond 60 weeks are included in the denominator. The first death from liver tumor occurred at 60 weeks.

The NTP bioassay doses for rats and mice have been converted into metabolized doses using the dose-metabolism relationship previously established. The metabolized dose, expressed in $mg/W^{2/3}/day$, where W is the body weight in kg, is assumed to be "equivalent" (equally potent) among species. The carcinogenic potencies (slopes) of PCE are calculated using the multistage model on the basis of "equivalent" doses and their corresponding tumor incidence rates. The slope is defined as the 95% upper confidence limit of the linear parameter in a multistage model. These slope estimates are presented in Table 4-6, and have been used for calculating the unit risk of PCE. It should be noted that these slope values are expressed in terms of "equivalent" doses. These values are only used in the intermediate steps of the derivation of the risk to humans due to 1 $\mu g/m^3$ of PCE in air. The slope estimates calculated on the basis of six different data sets are comparable, ranging from $3.64 \times 10^{-2}/(mg/W^{2/3}/day)$ to $1.21 \times 10^{-1}/(mg/W^{2/3}/day)$. It is possible to express slopes in terms of ppm ambient air concentrations by using the dose-metabolism relationship previously determined. Since such a conversion does not affect our derivation of risk to humans, it suffices to point out that slope values per ppm for mice are greater than those for rats because the rate of metabolism is greater in mice than in rats.

To calculate the human risk due to 1 $\mu g/m^3$ of PCE in air, it is necessary to estimate the corresponding amount metabolized in humans. As previously estimated, the amount metabolized is 3.33×10^{-5} mg when an individual is exposed to 1 $\mu g/m^3$ of PCE in air for 6 hours. As an approximation, the amount metabolized corresponding to a continuous (24 hours) exposure to 1 $\mu g/m^3$ of PCE in air is 3.33×10^{-5} mg x (24 hours/6 hours) = 1.33×10^{-4} mg/day, or 7.83×10^{-6} $mg/W^{2/3}/day$ for a 70-kg person.

An upper-bound estimate of the incremental risk due to 1 $\mu g/m^3$ of PCE

(i.e., unit risk) is calculated as 7.83×10^{-6} x slope. The risk estimates calculated on the basis of six different data sets (Table 4-6) are comparable, ranging from 2.85×10^{-7} to 9.47×10^{-7}, with a geometric mean equal to 5.78×10^{-7}. All of these values are comparable to our previous unit risk estimate of 4.8×10^{-7}, which is based on hepatocellular carcinomas in mice in the NCI (1977) gavage study.

4.7.2. Based on the Metabolized Dose Calculated from the PB-PK Model

For comparison, the inhalation unit risk for PCE is also calculated on the basis of metabolized dose derived from the PB-PK model, which has gained increasingly broad acceptance over the last few years as an effective tool for the study of the absorption, distribution, biotransformation, and elimination of chemicals in the body. However, due to the lack of data, some of the parameters that are used in the modeling (including the Michaelis-Menten kinetic parameters V_m and K_m) must be estimated from the same data that were used in the approach presented in the previous section. For this reason, the model so derived is not necessarily more reliable than that approach to predict the amount metabolized in the NTP bioassay. The metabolized dose ($mg/W^{2/3}/day$) in the NTP study, and the corresponding tumor incidence rate used in the risk calculation, are presented in Tables 4-7 and 4-8. Table 4-9 presents the resultant unit risk estimates calculated from the six different data sets. The risk calculated by this approach is also comparable to those calculated previously (Table 4-6), considering the fact that the risk calculated on the basis of mouse data is overestimated due to the underestimation of the metabolized dose by the PB-PK model (see section A.3.2.2. in Appendix A), in particular, when the risk estimates (in terms of $mg/W^{2/3}$) in the second columns of Tables 4-6 and 4-9 are compared. The risk estimates in the second columns are calculated directly from animal data, and thus do not reflect the uncertainty associated with the

TABLE 4-6. UNIT RISK ESTIMATES
CALCULATED ON THE BASIS OF DIFFERENT DATA SETS

Data set	Risk due to one unit ($mg/W^{2/3}$)[a] of urinary metabolized dose	Risk due to 1 $\mu g/m^3$ of PCE in air
Rat (leukemia):		
Males	1.21×10^{-1}	9.47×10^{-7}
Females	1.01×10^{-1}	7.91×10^{-7}
Mouse (liver carcinomas):		
Males	6.67×10^{-2}	5.22×10^{-7}
Females	3.64×10^{-2}	2.85×10^{-7}
Mouse (liver adenomas/ carcinomas):		
Males	1.08×10^{-1}	8.46×10^{-7}
Females	5.05×10^{-2}	3.95×10^{-7}
Geometric mean:	7.38×10^{-2}	5.78×10^{-7}

[a]The metabolized dose expressed in $mg/W^{2/3}$ (where W is body weight in kg) is assumed to be equivalent, i.e., equally potent, among species.

TABLE 4-7. NTP RAT METABOLIZED DOSE PREDICTED BY PB-PK MODEL AND THE CORRESPONDING MONONUCLEAR CELL LEUKEMIA INCIDENCE RATE

Sex	Exposure concentration (ppm)	Human equivalent metabolized dose ($mg/W^{2/3}/day$)[a]	Incidence rate
Males			
	0	0	28/50
	200	10.95	37/50
	400	13.68	37/50
Females			
	0	0	18/50
	200	10.16	30/50
	400	12.70	29/50

[a]Metabolized dose ($mg/W^{2/3}/day$) = d(mg/kg/day) x $W^{1/3}$, where d = 14.86 mg/kg/day and 18.57 mg/kg/day, respectively, for the 200 ppm and 400 ppm groups. The body weight W is 0.4 kg for male rats and 0.32 kg for female rats. For convenience, d is derived on the basis of a 0.35 kg rat, rather than calculated separately for 0.4-kg and 0.32-kg rats.

TABLE 4-8. NTP MOUSE METABOLIZED DOSE PREDICTED BY PB-PK MODEL AND THE CORRESPONDING LIVER TUMOR INCIDENCE RATE

Sex	Exposure concentration (ppm)	Human equivalent metabolized dose ($mg/W^{2/3}/day$)[a]	Incidence rate	
			Carcinomas	Carcinomas/adenomas
Males				
	0	0	7/49	16/49
	100	4.49	25/47	31/47
	200	6.55	26/50	40/50
Females				
	0	0	1/46	4/46
	100	4.27	13/42	17/42
	200	6.23	36/47	38/47

[a]Metabolized dose ($mg/W^{2/3}/day$) = d(mg/kg/day) x $W^{1/3}$, where d = 40.25 mg/kg/day and 58.75 mg/kg/day, respectively, for the 100 ppm and 200 ppm groups. The body weights are 0.0374 kg for male mice and 0.0322 kg for female mice. For convenience, d is derived on the basis of a 0.04-kg mouse.

TABLE 4-9. UNIT RISK ESTIMATES BASED ON PB-PK PREDICTED METABOLIZED DOSE

Data base	Risk due to one unit ($mg/W^{2/3}$) of total metabolites	Risk to humans due to 1 $\mu g/m^3$ of PCE in air	
		Method I[a]	Method II[b]
Rat (leukemia):			
Males	7.32×10^{-2}	3.40×10^{-6}	1.15×10^{-6}
Females	6.13×10^{-2}	2.85×10^{-6}	9.62×10^{-7}
Mouse (liver carcinomas)[c]:			
Males	1.42×10^{-1}	6.60×10^{-6}	2.23×10^{-6}
Females	7.74×10^{-2}	3.60×10^{-6}	1.21×10^{-6}
Mouse (liver adenomas/ carcinomas):			
Males	2.30×10^{-1}	1.06×10^{-5}	3.60×10^{-6}
Females	1.05×10^{-1}	4.88×10^{-6}	1.65×10^{-6}
Geometric mean:	1.03×10^{-1}	4.77×10^{-6}	1.61×10^{-6}

[a]Method I uses the human metabolized dose actually predicted by the PB-PK model. For a 70-kg person, the metabolized dose (at steady-state) corresponding to 1 $\mu g/m^3$ of PCE in air is 7.89×10^{-4} mg or 4.65×10^{-5} $mg/W^{2/3}$. The risk is calculated by multiplying 4.65×10^{-5} to the values in the second column.

[b]Method II uses the metabolized dose calculated from the direct observation of humans by Bolanowska and Golacka (1972). As discussed in section 4.6.1., the metabolites in the urinary excretion were estimated to be 7.83×10^{-6} $mg/W^{2/3}$ for a 70-kg person exposed to 1 $\mu g/m^3$ of PCE in air. If the urinary excretion is assumed to account for 50% of the total metabolites, the metabolized dose corresponding to 1 $\mu g/m^3$ of PCE in air would be 1.57×10^{-5} $mg/W^{2/3}$. This value is multiplied in the second column to obtain the risk. The assumption about the percentage of urinary metabolites in the total metabolites is based on the observation in rat and mouse studies where urinary metabolites account for more than 50% of total metabolites.

[c]The unit risks calculated on the basis of mouse data are somewhat over estimated because the metabolized dose was underestimated (see section A.3.2.2. in Appendix A for explanation).

dose-metabolism relationship for humans.

4.8. DISCUSSION

In calculating the dose-response relationship for PCE, the amount metabolized is considered to be an effective dose. The use of this surrogate effective dose may not eliminate the uncertainty associated with the low-dose extrapolation because the dose actually reaching the receptor sites may not be linearly proportional to the total amount metabolized, and the shape of the dose-response curve is still unknown. However, it seems reasonable to expect that the uncertainty with regard to the low-dose extrapolation would be somewhat reduced by the use of the metabolized dose because the metabolized dose better reflects the dose-response relationship, particularly in the high-dose region. To extrapolate from animals to humans, the metabolized dose per body surface area (which is proportional to $W^{2/3}$) is assumed to be equivalent (i.e., equally potent) among species. This assumption appears to be reasonable, since it is partially supported by the fact that the carcinogenic potency (i.e., slope) values are comparable between rats and mice when these values are expressed in terms of $mg/W^{2/3}$. It is interesting to note that the use of metabolic and kinetic information, when available, improves the accuracy of risk estimation, as evidenced by the fact that the PCE inhalation unit risk, calculated previously on the basis of gavage data, is shown to be a reasonable estimate when the newly released inhalation bioassay data are used.

The CAG has also employed the PB-PK model to calculate the amount metabolized in the NTP bioassay. This modeling technique provides insight into various assumptions used in the risk assessment. The most serious limitation for the PB-PK modeling is that the metabolism data reported in the literature do not represent periodic sampling over an interval of time. A detailed discussion dealing with PB-PK modeling is presented in Appendix A. The risk calculated by

this approach is also comparable to the previous risk estimates, considering the fact that the risk calculated on the basis of mouse data is overestimated due to the underestimation of the metabolized dose by the PB-PK model (see section A.3.2.2. in Appendix A); in particular, when one compares the risk estimates (expressed in terms of $mg/W^{2/3}$) in the second columns of Tables 4-6 and 4-9, which are calculated from the animal data.

4.9. SUMMARY OF QUANTITATIVE ASSESSMENT

This section updates the inhalation unit risk estimate for PCE, using data from the NTP (1985, draft) bioassay, which is the first lifetime inhalation study to show clear evidence of the carcinogenicity of PCE in both rats and mice. These data provide a basis for directly estimating the risk of PCE by the inhalation route, and an opportunity to test the reasonableness of the earlier risk estimate which was calculated using tumor incidence data from the NCI gavage cancer bioassay (1977) and the assimilated dose metabolized. The inhalation unit risk estimate reported in the 1985 Health Assessment Document for Tetrachloroethylene was $4.8 \times 10^{-7}/\mu g/m^3$.

The new unit risk estimate for inhalation also utilizes the metabolized dose-tumor incidence relationships derived from pertinent exposure concentration dose-metabolism relationship data in both animal and human experiments. The uncertainty associated with extrapolating from animals to humans is reduced by the consideration of actual human exposure concentration dose-metabolism relationship data where urinary TCA is taken as an index of the amount of PCE metabolized, in addition to the use of comparable animal data. It is of considerable interest to note that the air unit risk estimates calculated on the basis of animal inhalation carcinogenicity studies (rats and mice) are comparable to estimates derived previously from the oral gavage cancer data in mice, when a metabolized dose is used for the dose-tumor incidence relationships.

Furthermore, the risk per unit of metabolite is found to be comparable between rats and mice even though the risk for mice corresponding to a unit of ambient air concentration is higher than that for rats. The implication is that the cancer risk might be overestimated if it were assumed that PCE in the ambient air is equally potent among species. The upper-bound estimates of the incremental lifetime cancer risk due to lifetime exposure to 1 $\mu g/m^3$ of PCE in air are calculated by three methods for six sets of dose-tumor incidence data. The range of the six risk estimates under each of the three methods are as follows: (a) 2.9×10^{-7} to $9.5 \times 10^{-7}/\mu g/m^3$; (b) 2.9×10^{-6} to $1.1 \times 10^{-5}/\mu g/m^3$; and (c) 9.6×10^{-7} to $3.6 \times 10^{-6}/\mu g/m^3$.

The first range of estimates is calculated on the basis of metabolized doses that are derived directly from the single-exposure metabolism study, assuming that the total amount metabolized over the 72-hour period from the single exposure is comparable to the steady-state metabolized dose under the NTP multiple-exposure pattern. The second and third ranges of estimates are calculated on the basis of metabolized doses that are derived from the physiologically-based pharmacokinetic (PB-PK) model. Such models, which piece together the relevant physiological and biochemical information, can be useful tools for predicting absorption, distribution, biotransformation, and elimination of chemicals across species when sufficient data are available. However, until more data become available, the CAG recommends that the first range of of estimates be used to represent the inhalation risk for PCE, since there are uncertainties with parameters used in the PB-PK model that were estimated from the same empirical data set used in the direct estimation, and the model cannot be considered to give a better estimate. That is, the upper-bound estimate of the incremental cancer risk due to lifetime exposure of 1 $\mu g/m^3$ of PCE in air ranges from 2.9×10^{-7} to 9.5×10^{-7}. This range includes the previous estimate

of $4.8 \times 10^{-7}/\mu g/m^3$ derived from the NCI (1977) gavage data. Thus, the inhalation risk estimated from gavage carcinogenicity data is supported by the inhalation risk developed from the use of inhalation carcinogenicity data.

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APPENDIX A

PHYSIOLOGICALLY-BASED PHARMACOKINETIC MODELS AND THEIR APPLICATION IN THE QUANTITATIVE RISK ASSESSMENT OF PCE*

Physiologically-based pharmacokinetic (PB-PK) models have gained increasingly broad acceptance in recent years as effective tools for studying the absorption, distribution, biotransformation, and elimination of chemicals in the body. The most desirable characteristic of these models is their ability to piece together all of the relevant physiological and biochemical information to formulate a tool for predicting absorption, distribution, biotransformation, and elimination across species. In calculating the unit risk estimate for PCE, the PB-PK approach is used to supplement the risk calculations that are done on the basis of more directly estimated metabolized dose. The PB-PK model to be developed here is only applicable to inhalation exposures. The PB-PK approach is not considered to be superior to the approach that was used previously in Section 4.6.1. for the following reasons:

(1) Some of the parameters used in the PB-PK model must be estimated from the same set of data that is used in the crude approach. There are uncertainties with the parameters used in the model. Therefore, the amount of metabolites predicted by the PB-PK model is not necessarily more reliable.

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*Dr. Jerry Blancato of the Exposure Assessment Group provided valuable advice during the preparation of this Appendix, and Dr. Curtis Travis of the Oak Ridge National Laboratory provided valuable information regarding his work on PCE. Some of the data used in this Appendix are taken from a report entitled "Interpretation of Metabolic Data in Exposure Analysis," conducted by Oak Ridge National Laboratory and sponsored by the Exposure Assessment Group of the U.S. Environmental Protection Agency.

(2) Available data on the dose-metabolism relationship are primarily for rats, with limited data available for mice and humans. Therefore, the uncertainty associated with species conversion is somewhat reduced by the direct use of these data.

The use of the PB-PK approach in calculating the unit risk estimate for PCE has provided insight into the problems of using metabolism/kinetic data in quantitative assessment, and has pointed out a need for further research.

A.1. DESCRIPTION OF THE PB-PK MODEL FOR INHALED PCE

PCE in vapor form in air is readily absorbed through the lungs into blood by first-order diffusion processes. Pulmonary uptake of PCE is largely determined by the ventilation rate, duration and concentration of exposure, solubility in blood and body tissues, and metabolism. PCE is eliminated by pulmonary excretion and by metabolism (primarily in the liver). Figure A-1 depicts the PB-PK model used to simulate PCE distribution and metabolism over time. This model was used by Ramsey and Anderson (1984) to study the behavior of inhaled styrene. Abbreviations used in the figure are given in Table A-1.

The model in Figure A-1 is described by a system of differential equations which quantify the rate of change of PCE concentration in each tissue group over time. Pulmonary uptake and elimination are described by the following equation of mass balance of PCE entering and leaving the lungs:

$$Q_a(C_I - C_a) = Q_t(C_{art} - C_{ven})$$

This equation assumes a concentration equilibrium between arterial blood and alveolar air. By substituting the relationship $N = C_{art}/C_a$ into the equation above, we have

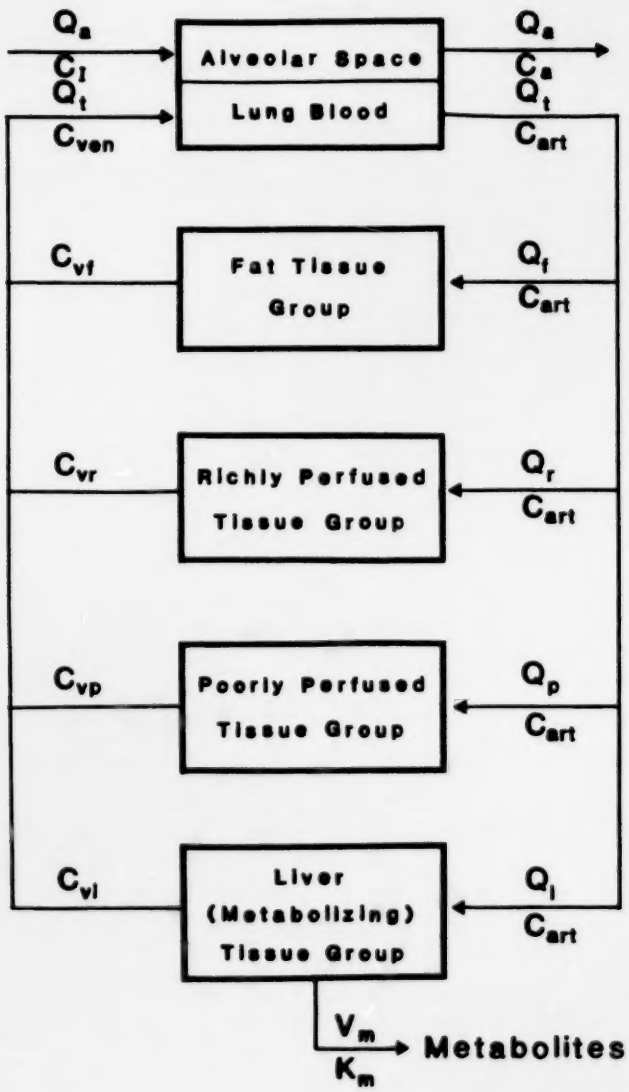

Figure A-1. Diagram of the physiologically-based pharmacokinetic model. Abbreviations are defined in Table A-1.

TABLE A-1. ABBREVIATIONS USED IN FIGURE A-1

Q_a:	Alveolar ventilation rate (L/min)
Q_t:	Cardiac blood output (L/min)
Q_ℓ:	Blood flow rate to liver (metabolizing) tissue group (L/min)
Q_f:	Blood flow rate to fat tissue group (L/min)
Q_r:	Blood flow rate to richly-perfused tissue group (L/min)
Q_p:	Blood flow rate to poorly-perfused tissue group (L/min)
V_ℓ, V_f, V_r, and V_p:	Volumes of tissue groups (L) corresponding, respectively, to liver, fat, richly-perfused, and poorly-perfused tissue groups
N:	Blood:air partition coefficient
P_ℓ, P_f, P_r, and P_p:	Tissue:blood partition coefficient corresponding, respectively, to liver, fat, richly-perfused, and poorly-perfused tissue groups
V_m:	Maximum velocity of metabolism (mg/min)
K_m:	Michaelis constant (mg/L blood)
A_m:	Amount metabolized (mg)
C_{art}:	Concentration in arterial blood (mg/L)
C_{ven}:	Concentration in venous blood (mg/L)
C_ℓ, C_f, C_r, and C_p:	Concentrations in tissue groups corresponding, respectively, to liver, fat, richly-perfused, and poorly-perfused tissue groups
C_I:	Concentration in inhaled air (mg/L)
C_a:	Concentration in alveolar air (mg/L)

$$C_{art} = (Q_a C_I + Q_t C_{ven}) / (Q_t + Q_a/N)$$

where venous blood concentration is given by

$$C_{ven} = (Q_\ell C_\ell/P_\ell + Q_f C_f/P_f + Q_r C_r/P_r + Q_p C_p/P_p) / Q_t$$

The metabolism of PCE occurs mainly in the liver, with a rate of metabolism characterized by the Michaelis-Menten type equation

$$\frac{dA_m}{dt} = (V_m C_\ell/P_\ell) / (K_m + C_\ell/P_\ell)$$

The PCE concentrations in each of the four tissue groups are described by the following equations:

Metabolism tissue group:

$$V_\ell \frac{dC_\ell}{dt} = Q_\ell (C_{art} - C_\ell/P_\ell) - \frac{dA_m}{dt}$$

Non-metabolism tissue groups:

$$V_i \frac{dC_i}{dt} = Q_i (C_{art} - C_i/P_i)$$

where subscript i represents fat, richly-perfused, and poorly-perfused tissue groups.

A.2. PARAMETERS USED IN THE MODEL

Table A-2 presents the physiological and biochemical parameters used in the model. How these parameters were obtained is described below.

TABLE A-2. PHYSIOLOGICAL AND BIOCHEMICAL PARAMETERS USED IN THE MODEL

Parameters	Rats	Mice	Humans
Body weight (kg)	0.35	0.04	70
Alveolar ventilation rate			
Q_a	0.083	0.018	3.421
Blood flow rate			
Q_t	0.104	0.0225	4.31
Q_ℓ	0.0389	0.00836	1.607
Q_f	0.00920	0.00198	0.387
Q_r	0.0434	0.00940	1.800
Q_p	0.0126	0.00272	0.506
Tissue volume			
V_ℓ	0.0140	0.00160	0.8000
V_f	0.0315	0.00359	6.2970
V_r	0.0150	0.00199	3.4980
V_p	0.2550	0.02920	51.085
Partition coefficient			
N	18.9	16.9	10.3
P_ℓ	3.719	4.159	3.719
P_f	108.994	121.893	108.994
P_r	3.719	4.159	3.719
P_p	1.058	1.183	1.058
V_m	0.00586	0.00138	0.2004
K_m	2.9378	2.9378	2.9378

A.2.1. Physiological Parameters

These parameters are taken from Ramsey and Anderson (1984), with adjustment for the difference in body weight. This adjustment is performed by assuming that within a given species the ventilation and blood flow rates are proportional to the two-thirds power of body weight, and the volume of each tissue group is directly proportional to the body weight. For example, the alveolar ventilation rate for a 0.35-kg rat is calculated by

$$Q_a = 0.075 \times (0.35/0.3)^{2/3} = 0.083 \text{ L/min}$$

where 0.075 L/min is the ventilation rate for a 0.30-kg rat that is given in Ramsey and Anderson (1984).

A.2.2. Partition Coefficients

The parameters for rats and mice were provided by Dr. Curtis Travis of the Oak Ridge National Laboratory, which is the contractor for the EPA project, "Interpretation of Metabolic Data in Exposure Analysis," sponsored by the Exposure Assessment Group, Office of Health and Environmental Assessment. It is our understanding that these parameters were obtained by Dr. Melvin E. Anderson of the Air Force Medical Research Laboratory at Wright-Patterson Air Force Base, using the equilibrium vial technique. The tissue:blood concentration ratio was calculated by taking the ratio of two concentration ratios, air/blood and air/homogenate tissue. The tissue:blood coefficients for humans are assumed to be identical to those of rats.

A.2.3. Metabolism Rate Constants, V_m and K_m

Rats: The constants V_m and K_m were estimated by least-square optimization using the system of differential equations and data from a metabolism study by Pegg et al. (1979). As presented in Table 4-1, the amounts metabolized over 72

hours for a 0.25-kg rat exposed to PCE at 10 ppm and 600 ppm for 6 hours were recorded to be, respectively, 0.467 mg and 9.11 mg. These two data points (72 hours, 0.467 mg) and (72 hours, 9.11 mg), were used to fit the system of differential equations, described previously, with V_m and K_m as unknown parameters. The resultant estimates are V_m = 0.004685 mg/min and K_m = 2.9378 mg/L. For the NTP bioassay rats, which had approximately 0.35 kg body weight, the estimates are $V_m = 0.004685 \times (0.35/0.25)^{2/3} = 0.00586$ mg/min, with K_m the same, independent of body weight.

Mice: An approach similar to the one used for rats cannot be used for mice because there is only one data point for mice; the amount metabolized over 72 hours was 0.356 mg when mice were exposed to 10 ppm of PCE for 6 hours (see Table 4-1). To derive V_m and K_m for mice, we assume that the ratio of V_m between two species is equal to the two-thirds power of the ratio of their body weights, and that K_m is the same across species. For a 0.04-kg mouse,

$$V_m = 0.004685 \times (0.04/0.25)^{2/3} = 0.00138 \text{ mg/min}$$

and

$$K_m = 2.9378 \text{ mg/L}$$

These results are presented in Table A-2.

Humans: The metabolism constants for humans were calculated in a similar way as the constants for mice. The results are presented in Table A-2.

A.3. RESULTS AND APPLICATIONS TO RISK ASSESSMENT

A.3.1. Results Under a Single Exposure Scenario

Figure A-2 describes the amount metabolized over an 80-hour (4,680-min) period for a 0.35-kg rat exposed to 200 ppm and 400 ppm of PCE for 6 hours.

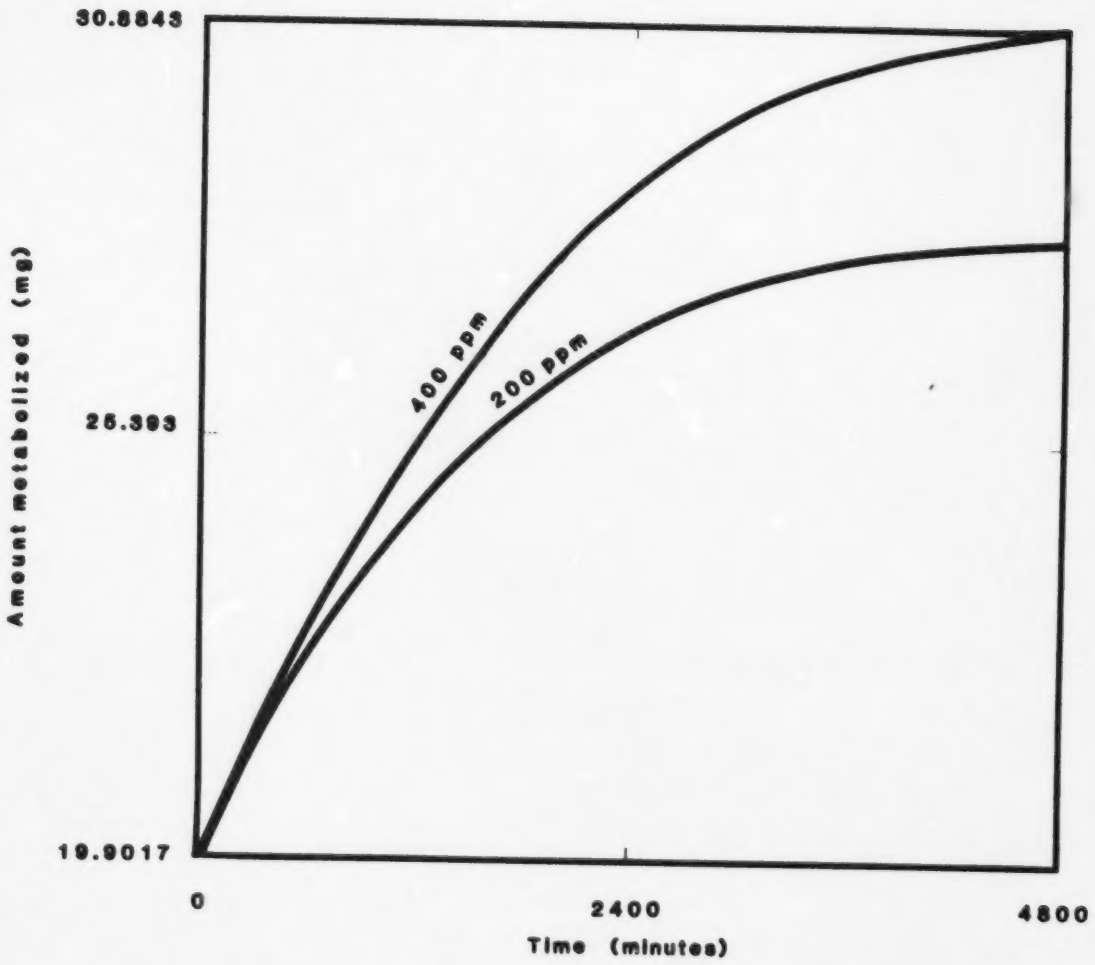

Figure A-2. Amount metabolized for a 0.35-kg rat exposed to 200 ppm and 400 ppm of PCE for 6 hours.

From this figure, it is seen that the curve for the 200-ppm group reaches asymptotic value at about 72 hours, whereas the curve for the 400-ppm group approaches its asymptotic value at or beyond 80 hours. Thus, the assumption that the metabolites collected over the 72-hour period represent the total amount metabolized is justified only for the 200-ppm group and represents, at most, a very crude approximation for the higher dosed group. Figures A-3 through A-7 depict blood and tissue concentration curves over an 80-hour (4,680-min) period. These curves indicate that PCE is rapidly removed in all of the tissue groups except the fat tissue group.

A.3.2. Results Under the NTP Multiple Exposure Scenario

Animals in the NTP bioassay were exposed to PCE 6 hours/day, 5 days/week. It would be of interest to investigate whether the amount metabolized reaches steady state during the weekday exposure period and whether the metabolites are eliminated over the weekend.

A.3.2.1. Results for Rats--From Table A-3, it is seen that the amount metabolized per day reaches steady state quickly at the second day of exposure. The steady-state values are about 5.2 mg/day and 6.5 mg/day, respectively, for the 200-ppm and 400-ppm dosed groups. Although the amounts metabolized in the first and the last two days of the week are less than the steady-state values, these values increased from the first week to the second week, and thus are expected to increase to larger values than the values presented in Table A-3. As an approximation, the steady-state values are taken as the effective daily dose rate for the risk calculation (see Table 4-7).

A.3.2.2. Results for Mice--The results for mice are more uncertain than for rats because the metabolism constants V_m and K_m must be converted from those for rats. Using the converted V_m and K_m for mice, the amount metabolized for a 0.025-kg mouse that is exposed to 10 ppm of PCE for 6 hours is predicted to be

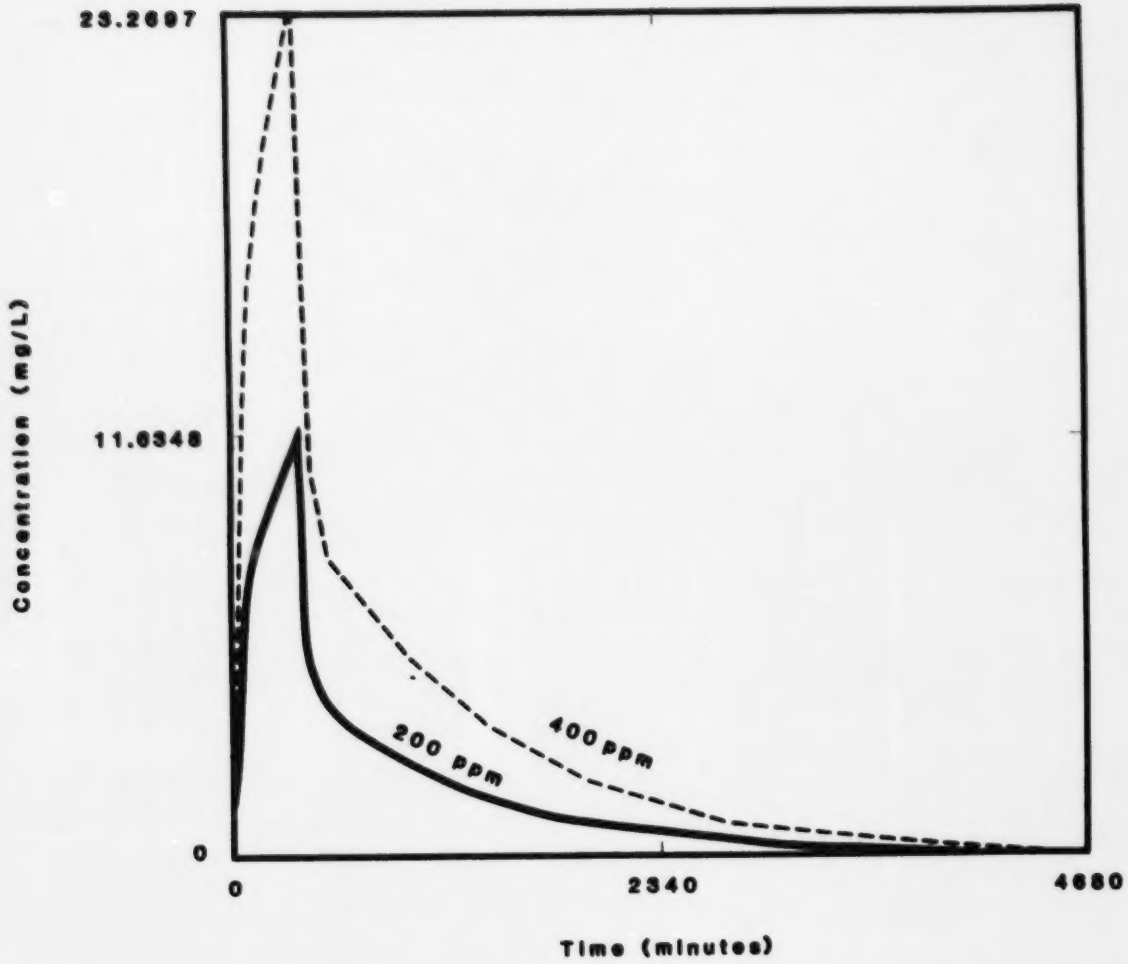

Figure A-3. PCE concentration (mg/L) in venous blood for a 0.35-kg rat exposed to PCE at 200 ppm and 400 ppm for 6 hours.

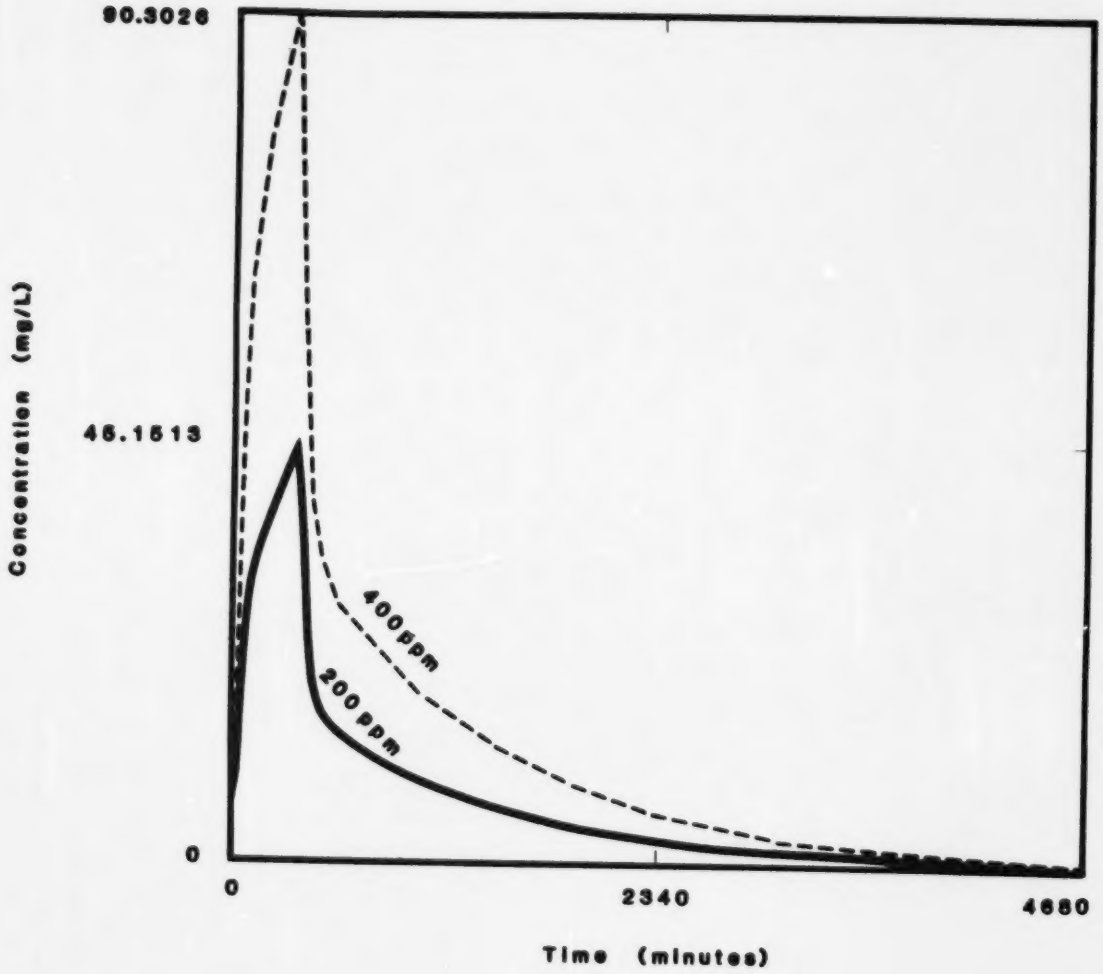

Figure A-4. PCE concentration (mg/L) in liver (metabolizing) tissue for a 0.35-kg rat exposed to PCE at 200 ppm and 400 ppm for 6 hours.

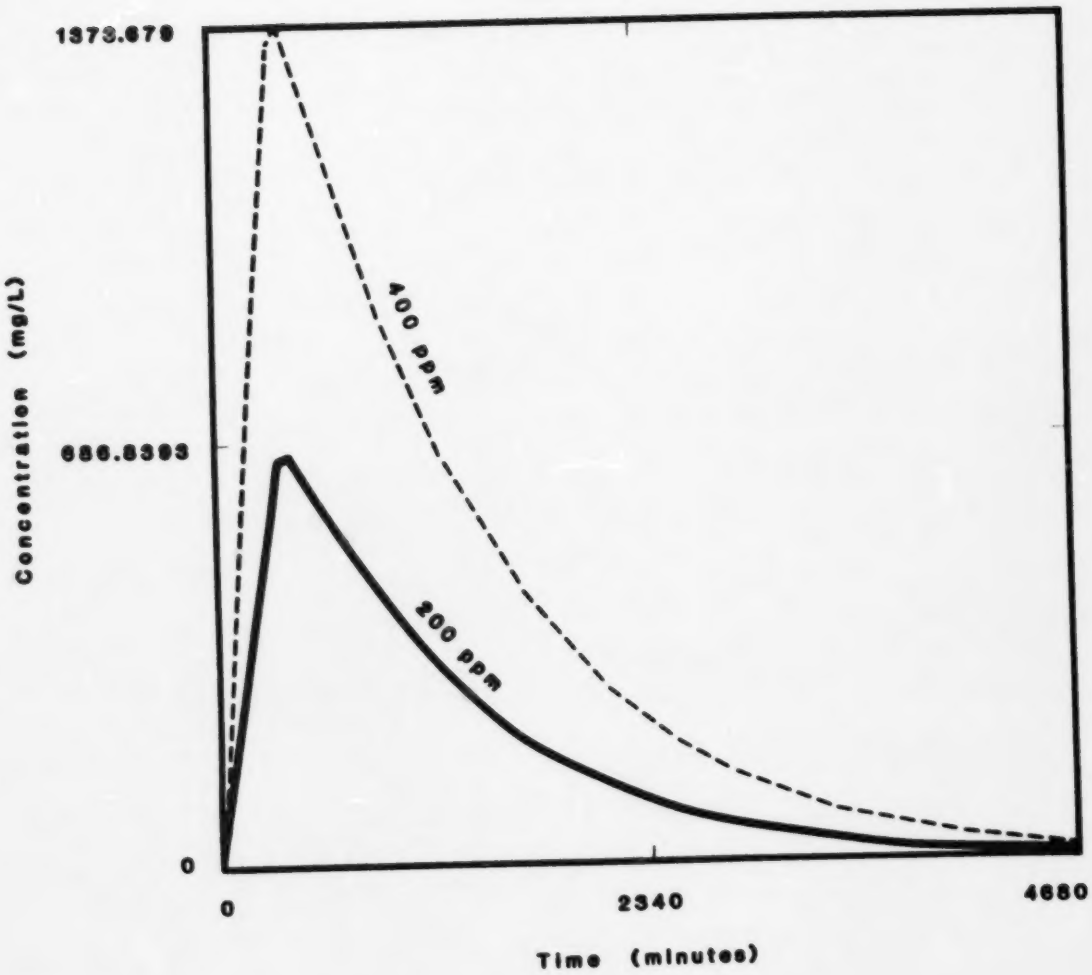

Figure A-5. PCE concentration (mg/L) in fat tissue for a 0.35-kg rat exposed to PCE at 200 ppm and 400 ppm for 6 hours.

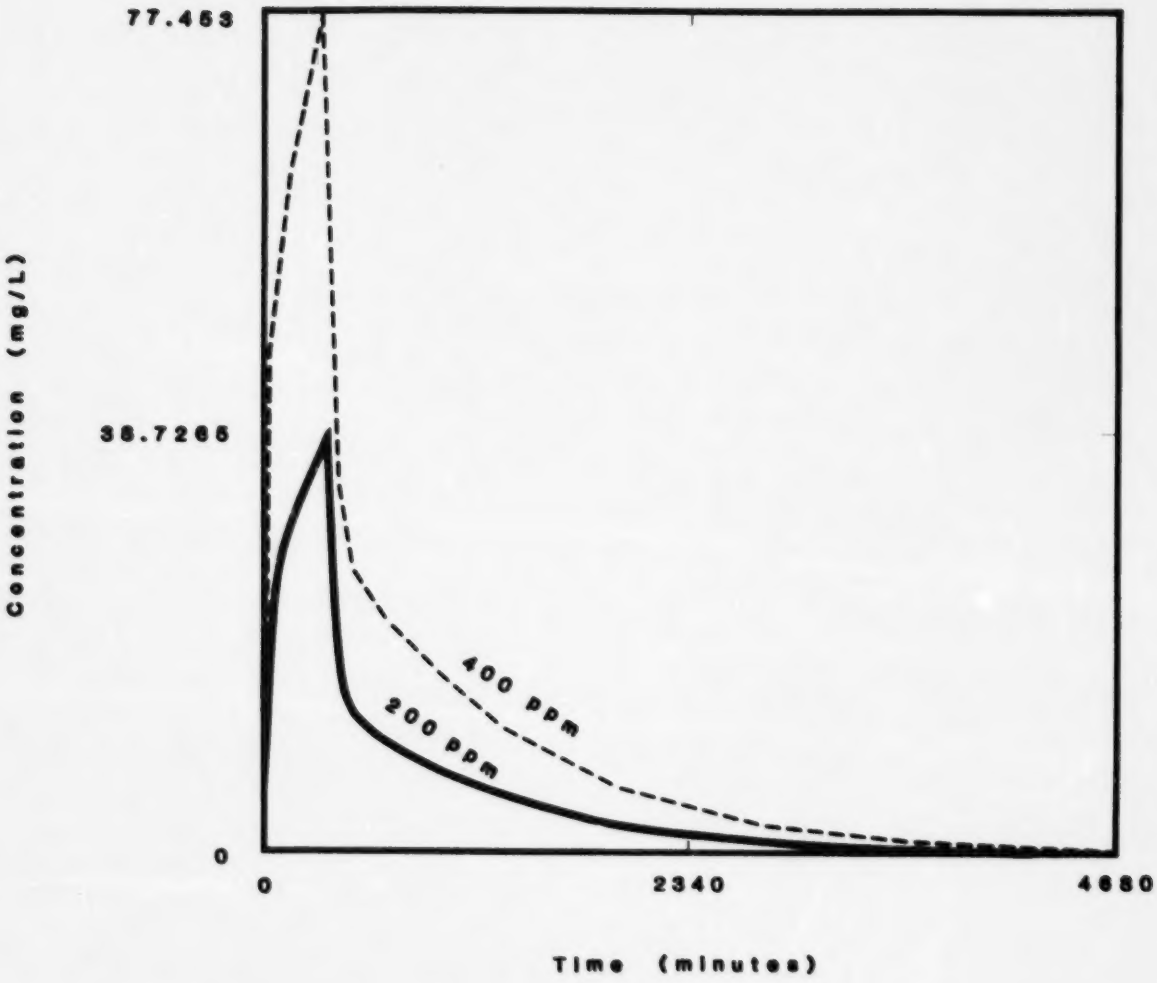

Figure A-6. PCE concentration (mg/L) in richly perfused tissue for a 0.35-kg rat exposed to PCE at 200 ppm and 400 ppm for 6 hours.

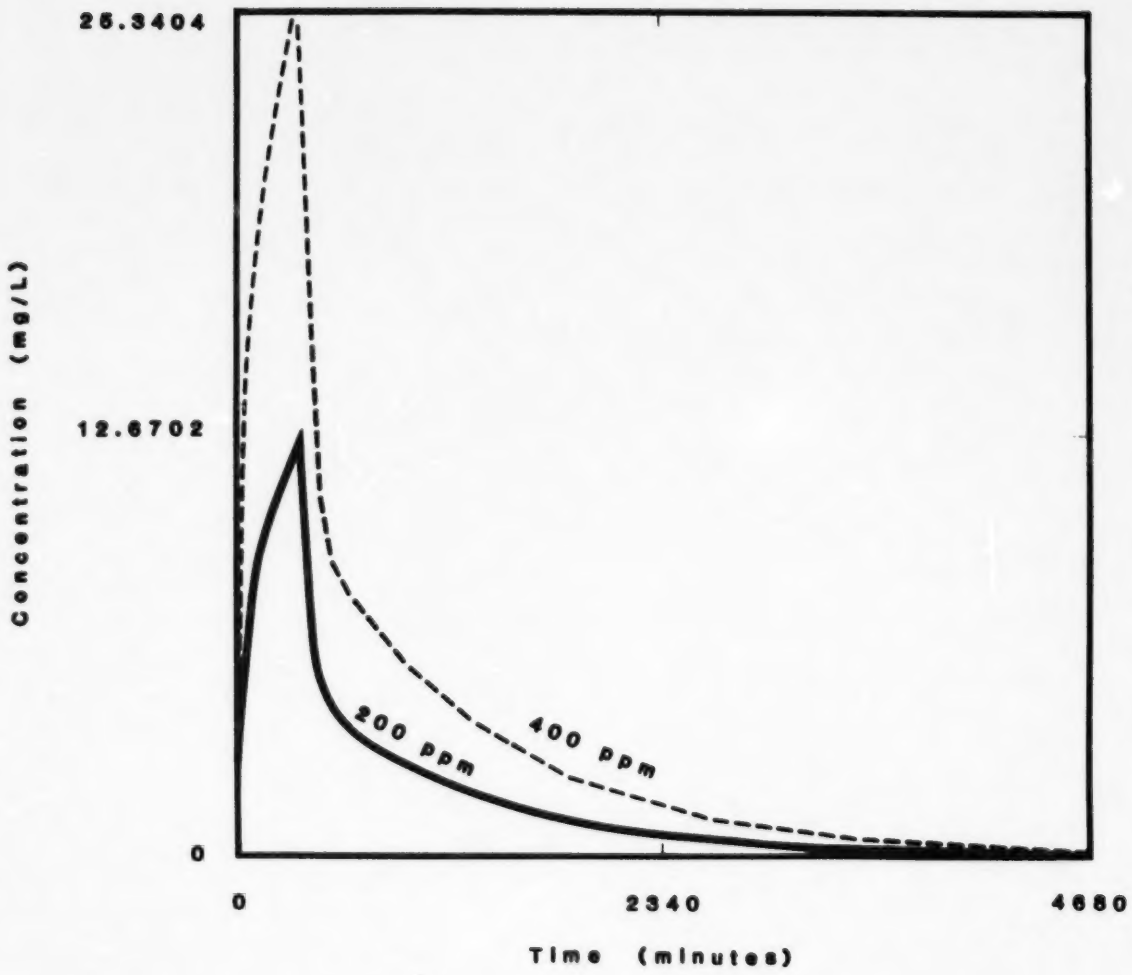

Figure A-7. PCE concentration (mg/L) in poorly perfused tissue for a 0.35-kg rat exposed to PCE at 200 ppm and 400 ppm for 6 hours.

TABLE A-3. DAILY METABOLITES (mg)
FOR A 0.35-kg RAT
EXPOSED TO PCE AT THE NTP BIOASSAY EXPOSURE PATTERN

Week	Day	Exposure concentration (mg)	
		200 ppm	400 ppm
1	Mon.	4.575	5.946
	Tues.	5.017	6.346
	Wed.	5.134	6.439
	Thur.	5.166	6.467
	Fri.	5.175	6.474
	Sat.	2.260	3.791
	Sun.	0.670	1.409
2	Mon.	4.614	5.987
	Tues.	5.032	6.352
	Wed.	5.137	6.441
	Thur.	5.167	6.467
	Fri.	5.175	6.475
	Sat.	2.260	3.792
	Sun.	0.670	1.413

0.10 mg, which is only one-third of that observed in the metabolism study by Pegg et al. (1979). The reason for the underestimation becomes obvious when one examines the following calculation. For a 0.025-kg mouse, the alveolar ventilation rate (given by Ramsey and Anderson, 1984) is 0.0132 L/min. At 10 ppm (i.e., 0.06908 mg/L), 6-hour exposure, the total uptake (including the amount metabolized) is no more than

$$0.0132 \text{ L/min} \times 0.06908 \text{ mg/L} \times 6 \text{ hr} \times 60 \text{ min/hr} = 0.328 \text{ mg}$$

which is even less than the metabolized value of 0.356 mg observed by Pegg et al. (1979). Thus, the underestimation is probably due to use of undervalued physiological parameters; in particular, the ventilation rate. Although it is possible to adjust the ventilation rate and other parameters in the model, we choose not to do so because the difference between the observed and predicted metabolized dose is not large. However, one should keep in mind that the risks calculated on the basis of mouse data (using the PB-PK model) may be overestimated due to the underestimation of the metabolized dose. Table A-4 presents the amount metabolized for mice.

The NTP mice reached steady state quickly, with steady-state values at 0.74 mg/day and 1.07 mg/day, respectively, for the 100-ppm and 200-ppm dosed groups. However, the mice appeared to metabolize faster than the rats, as evidenced by the fact that the metabolized dose almost reached steady state on the first day of exposure. Furthermore, the mice also eliminated faster than the rats, as evidenced by the fact that the metabolites were almost totally removed over the nonexposed weekend. Therefore, it is more appropriate to use as the daily dose, the averaged amount metabolized over 7 days. That is, the effective doses for the 100-ppm and 200-ppm dosed groups are respectively 0.54

TABLE A-4. DAILY METABOLITES (mg)
FOR A 0.04-kg MOUSE
EXPOSED TO PCE AT THE NTP BIOASSAY EXPOSURE PATTERN

	Exposure concentration (mg)	
Day	100 ppm	200 ppm
Mon.	0.710	1.027
Tues.	0.740	1.064
Wed.	0.742	1.067
Thur.	0.743	1.067
Fri.	0.743	1.067
Sat.	0.084	0.175
Sun.	0.006	0.013

mg/day and 0.78 mg/day. These effective daily doses have been used in the risk calculation (see Table 4-8).

A.3.3. Results for Human Exposure

In the previous risk calculation (sections 4.5 and 4.6.1), the study by Bolanowska and Golacka (1972) was used to estimate the human dose-metabolism relationship. In that study, five subjects were exposed to 390,000 ug/m^3 of PCE for 6 hours. The metabolites in the urinary excretion were estimated to be 13 mg. Assuming that the urinary excretion accounts for 50% of the total metabolites, one would expect that the PB-PK model predicts 26 mg of the total metabolites. This assumption is based on the observation from rat and mouse studies that urinary metabolites accounted for at least 50% of total metabolites. The amount of metabolites actually predicted by the PB-PK model under the above exposure scenario is 64.34 mg, which is about 2.5 times greater than the expected value of 26 mg. Therefore, if the Bolanowska and Golacka study is considered adequate, the PB-PK model may somewhat overpredict the magnitude of human metabolites and thus lead to an overestimation of human risk by a factor of about 2 to 3. However, it is remarkable that the PB-PK model could predict with such accuracy despite the fact that the metabolism for humans must be estimated from that of rats.

To calculate the unit risk for humans, it is necessary to calculate the amount metabolized associated with a continuous (24-hour) exposure to 1 ug/m^3 of PCE in air. Table A-5 presents the consequences of the PCE exposure over a 2-week (14-day) period during which the steady state is reached. The steady-state value of the amount metabolized is 7.9 mg/day. This value was used to calculate the unit risk of PCE in Table 4-9.

Table A-6 presents the consequences of continuous (24 hours/day) exposure to PCE at higher concentrations. The values presented in Table A-6 represent

TABLE A-5. AMOUNT METABOLIZED PER DAY FOR A 70-kg PERSON EXPOSED TO 1 $\mu g/m^3$ OF PCE IN AIR

Day	Amount of metabolite (mg) at end of each day
1	4.36×10^{-4}
2	6.108×10^{-4}
3	6.43×10^{-4}
4	6.92×10^{-4}
5	7.25×10^{-4}
6	7.47×10^{-4}
7	7.62×10^{-4}
8	7.71×10^{-4}
9	7.78×10^{-4}
10	7.82×10^{-4}
11	7.85×10^{-4}
12	7.87×10^{-4}
13	7.88×10^{-4}
14	7.88×10^{-4}

TABLE A-6. STEADY-STATE METABOLIZED DOSE (mg/day)
FOR A 70-kg PERSON
CONTINUOUSLY EXPOSED TO PCE IN AIR

Ambient air concentration (ppm)	Amount metabolized (mg)
150	221.96
125	211.71
100	197.82
75	178.02
50	147.76
25	96.89
1	5.37

the amount metabolized per day 14 days after first exposure; these values are considered to be steady-state values.

A.4. COMMENTS

As demonstrated in this Appendix, the PB-PK model can be a useful tool for quantitative risk assessment. It provides not only the estimation of the metabolized dose in the NTP bioassay, but also an insight into the validity of various assumptions that must be made in risk assessment, such as the validity of using data from a single-exposure experiment in place of multiple-exposure bioassay data. For the PCE PB-PK modeling, we find the following data to be desirable:

(1) The tissue concentrations and the amount metabolized over time are useful. For PCE, only metabolism data at 72 hours are available. The measurements at several time points and at different exposure concentrations are necessary for estimating the parameters and verifying the adequacy of the model constructed.

(2) The physiological and biochemical parameters for the animals used in a metabolism/kinetic study are useful. Although the physiological parameters for most species are known to be within certain ranges, the use of exact parameter values for the animals could greatly improve the accuracy of the prediction by the model. We feel that work should be initiated to develop a methodology that better quantifies key metabolic parameters in humans.